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Water quality restoration by harvesting mixed culture microalgae using *Moringa oleifera*

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Received 5 December 2019; Revised 17 February 2020; Accepted 1 March 2020

University Grants Commission, Grant/Award Number: 201516-RGNF-2015-17-SC-UTT-14868

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DOI: 10.1002/wer.1322

Abstract

Excessive growth of microalgae due to use of fertilizers, detergents, and discharge of domestic waste results in the eutrophication and degradation of the water quality of lakes and ponds. The present study aimed to improve the quality of pond water by harvesting mixed cultures of microalgae grown in a pond using *Moringa oleifera* (MO) extract as a coagulant. The study comprises evaluating the effects of coagulant dose, pH, mixing time, mixing rate, settling time, temperature, and algal biomass concentration on nutrient and microalgae cell removal. The pond water quality restoration was assessed by characterization of raw and MO-treated water. The MO was found to be very effective for mixed culture microalgae harvesting with flocculation efficiency of 92.97% at dose of 15 ml/L of MO extract, pH 8, mixing time 5 min, mixing rate 30 rpm, and 20-min settling time. The results have shown significant improvement in pond water quality with reduction in turbidity from 388.16 ± 48.23 to 8.39 ± 1.0 NTU, chlorophyll-a from 635.01 ± 86.20 to 15.03 ± 1.5 mg/m^3, total nitrogen (TN) from 20.37 ± 4.64 to 12.10 ± 1.19 mg/L, and total phosphorus (TP) from 2.05 ± 0.10 to 1.61 ± 0.24 mg/L. © 2020 Water Environment Federation

Practitioner points

- Production of biomass and water quality improvement of the pond at the same time
- MO extract shows better removal efficiency at a lower dose with fast kinetics
- Cost-effective flocculant saves time and energy during flocculation
- Common bioflocculant to remove both microalgae and nutrients.

Key words

eutrophication; flocculation; microalgae; *Moringa oleifera*; water quality restoration

Introduction

Ponds and lakes have a very significant role in the sustainable development of the country. Their environmental and socio-economic functions such as a source of drinking water, boosting groundwater, flood controller, supporting bio-diversity, and use for recreation make them a precious resource that needs to be conserved and managed properly. Increasing population and demand for freshwater, particularly in rural areas where the source of drinking water is limited, has necessitated proper water quality management of village ponds and lakes. India gets over 75% of the annual rainfall in the four rainy months of June to September, leading to an average annual rainfall of 119 mm but precipitation is exceptionally unevenly distributed with reference to time and space in the country. The ponds and lakes are usually created in the depressions by receiving runoff from the nearby catchment area. The surface runoff often carries nutrients from the fields to the ponds and lakes and causes eutrophication. Today, most lakes and ponds are affected by eutrophication.

For dealing with eutrophication, researchers have come up with many suggestions including diversion of excess nutrients, altering nutrient ratios, physical mixing, shading water bodies with opaque liners, and application of potent algaecides (Boyd & Tucker, 2012; Chislock, Doster, Zitomer, & Wilson, 2013; Downing, Watson,

& McCauley, 2001; Edmondson, 1970; Huisman et al., 2004). Patidar (2009) discussed nutrient control and various in situ remediation approaches for the restoration of ponds and lakes. Harvesting of microalgae results in eutrophication control and production of concentrated algae biomass. Various microalgae harvesting techniques such as centrifugation, membrane filtration, coagulation/flocculation, and flotation are used for harvesting microalgae. Coagulation has advantages like fast and easy technique, applicable on large scale for a vast range of species, less cell damage, and less energy requirements. If bioflocculants are used, coagulation is an inexpensive and sustainable method also, while other techniques are expensive, time-taking, and species-specific and even result in toxicity to harvested biomass. Singh and Patidar (2018) reviewed various microalgae harvesting techniques and reported coagulation to be a more beneficial technique than other costly and time-consuming techniques. The use of natural organic coagulants over other coagulants adds further advantages to the coagulation method. The natural organic coagulants are environmental friendly, nontoxic, noncorrosive, and biodegradable (Abdul Hamid et al., 2014; Ahmad, Mat Yasin, Derek, & Lim, 2011; Teixeira, Kirsten, & Teixeira, 2012). Earlier polyvalent salts of aluminum and iron have been used for coagulating drinking water impurities. Papazi, Makridis, and Divanach (2009) screened twelve different salts and concluded that chloride and sulfate salts of aluminum and iron were most effective in coagulating *Chlorella minutissima* from its culture medium. Wyatt et al. (2012) also recovered *Chlorella zofingiensis* from the culture medium with >90% efficiency using ferric chloride at optimum dosage and pH. Yang et al. (2018) developed the concept of coagulant recovery and recycle Al^{3+} from the flocculated *Scenedesmus acumi-natus* biomass and reported 50% reduction in chemical cost after 5-time recycling. Although good removal efficiency is achieved by using metal salts, the problems like cell lysis and cell discoloration hinder use of metal salts as a coagulant. Researchers had used various organic and synthetic polymers for microalgae harvesting. Tenney, Echelberger, Schuessler, and Pavoni (1969) used different polymers for recovery of microalgae and observed that only cationic polymer was effective in coagulation of microalgae and no coagulation was observed with the anionic and nonionic polymers. Biopolymers, such as cassia, starch, chitosan, guar gum, and tamarind kernel polysaccharide, were studied extensively for their potential to harvest microalgae (Banerjee et al., 2014). In addition, polymers produced by structural modification such as grafted guar gum, grafted tamarind kernel polysaccharide, hydrolyzed polyacrylamide-grafted tamarind kernel polysaccharide, cationic guar gum, starch-grafted polyacrylamide, crystalline nanocellulose modified with 1-(3-aminopropyl) imidazole, cationic cassia gum, cationic inulin, poly-L-lysine, cationic corn, and cationic locust bean gum have been elaborately studied for the flocculation of microalgae (Kumar, Banerjee, Kumar, & Jagadevan, 2019; Noh, Kim, Lee, Ryu, & Kang, 2018; Pugazhendhi et al., 2019; Qiu et al., 2019; Rahul, Kumar, Jha, & Sen, 2015). Knuckey, Brown, Robert, and Frampton (2006) reported >80% recovery of marine microalgae using nonionic polymer (Magnafloc LT) by adjusting the pH. Natural polymer such as chitosan removed >99% *Chlorella* sp. at a relatively low dose and small sedimentation time (Ahmad et al., 2011). Behera and Balasubramanian (2019) have used plant extracts of neem, cactus, drumstick, and waste fruit peels of orange, pomegranate, and banana for harvesting the mixed microalgal consortium. Magnetophoretic technique has also emerged as an energy-efficient and time-saving technique for microalgal harvesting (Wang, Yang, Hong, & Hou, 2016). Magnetite particles are often coated with cationic polymers such as poly (diallyldimethylammonium chloride) (PDADMAC), polyethylenimine (PEI), or natural cationic biopolymers (e.g., chitosan). Besides magnetite, other magnetic materials such as aminoclay–nanoscale zerovalent iron (nZVI) composites were also used for algal separation (Ge, Agbakpe, Zhang, & Kuang, 2015). Even bare iron (II, III) oxide (Fe_3O_4) and yttrium iron oxide ($Y_3Fe_5O_{12}$) magnetic nanoparticles act as efficient flocculants (Zhu, Hiltunen, & Li, 2017).

Moringa oleifera (MO) is one of the most cultivated species of Moringaceae family (Jahn, 1988). It is abundant in the tropic and subtropic regions of India, Pakistan, Bangladesh, and Afghanistan (Talreja, 2010). *Moringa oleifera* is commonly referred to as the miracle tree because of the multipurpose uses of the various plant parts. Its leaves, seeds, and flowers can be used in food, phytochemicals, medicines, cosmetics, lubricants, water, and wastewater treatment, etc. (Amaglo et al., 2010; Anwar, Latif, Ashraf, & Gilani, 2007; Gassenschmidt, Jany, Bernhard, & Niebergall, 1995; Ghebremichael, Gunaratna, Henriksson, Brumer, & Dalhammar, 2005; Kleiman, Ashley, & Brown, 2008; Mani, Jaya, & Vadivambal, 2007; Mendieta-Araica, Spörndly, Reyes-Sánchez, & Spörndly, 2011; Pandey, Pradheep, Gupta, Nayar, & Bhandari, 2010; Rashid, Anwar, Moser, & Knothe, 2008). Exhaustive literature reviews have documented the usefulness of the MO seeds for water treatment for adsorption or coagulation. Earlier researches have shown its ability to remove turbidity, hardness, heavy metals, and microalgae cells (Abdul Hamid et al., 2014; Knuckey et al., 2006; Muyibi & Evison, 1995a). The *M. oleifera* as compared with conventional chemical coagulants is cost-effective, easily available, biodegradable, and eco-friendly, produces low sludge volume, and contributes no harmful by-products (Abdul Hamid et al., 2014; Baharuddin et al., 2016). In view of these advantages, *M. oleifera* seed extract can be an excellent alternative to chemical coagulants with potential applications in water treatment in developing countries (Ndabigengesere & Narasiah, 1998). The seeds from different origins and lots have almost the same efficiency (Teixeira & Teixeira, 2017). Abdul Hamid et al. (2014) evaluated *Chlorella* sp. removal by using MO seed derivatives and observed that seed protein powder was better than seed powder in terms of removal of microalgae. MO protein resulted in >90% removal of *Chlorella* sp. within 20 min of settling time. Barrado-Moreno, Beltran-Heredia, and Martín-Gallardo (2016) evaluated cationic starch (Optifloc), *M. oleifera* extract, and several modified tannins (Acquapol, Silvafloc, and Tanfloc) for removal of microalgae (*Chlorella*, *Microcystis*, *Oocystis*, and *Scenedesmus*). Among them, *M. oleifera* was found to be the better coagulant for the removal of the microalgae. Baharuddin et al. (2016) compared removal of *Nannochloropsis oculata* using MO after oil extraction (MOAE)

Table 1. Variations in pH, nitrate, TN, and conductivity of water due to varying MO extract dosing

S. NO.	DOSE (ML/L)	DOSE (MG/L)	PH	CONDUCTIVITY (MS/CM)	TURBIDITY (NTU)	ORTHO PHOSPHATE (MG/L)	TOTAL PHOSPHOROUS (MG/L)	TOTAL NITROGEN (MG/L)
1.	0	0	4.71	2.21	0.405	0	0	0
2.	5	278.4	5.03	307	109	0.204	0.57	7.56
3.	10	556.8	5.12	579	238	0.348	1.04	15.15
4.	15	835.2	5.19	852	319	0.572	1.76	22.78
5.	20	1,113.6	5.28	1,112	447	0.724	2.31	29.33
6.	25	1,392	5.40	1,376	528	0.932	2.98	37.60

and without oil extraction (MOWE) and reported that MOAE had flocculation efficiency of 93.77% at pH 7 with long settling time of 150 min at 5,000 mg/L dosage, whereas MOWE had flocculation efficiency of 70.56% at same pH, settling time 90 min, and 4,000 mg/L dosage. Teixeira and Teixeira (2017) evaluated the flocculation efficiency of *Chlorella vulgaris* harvested by *M. oleifera* seed under different forms: flour, seed cake, and extracts of flour and cake. The use of flour extracts and cake extracts had the best cost–benefit ratio with increased removal efficiency when the extract was made with saline and seawater (Teixeira & Teixeira, 2017). Ali, Mustafa, and Saleem (2018) compared natural coagulants such as de-oiled jatropha curcas cake, *M. oleifera*, conocarpus erectus, and neem seeds with alum as a chemical coagulant. The techno-economic analysis showed that the powdered neem and MO seeds were most economical in terms of mixed microalgae harvesting with cost US$ 0.0068/L and US$ 0.0052/L, respectively.

Moringa oleifera seed utilization is not new, and it has been in use for a long time. Earlier, its usage was mainly focused on drinking water treatment (Teixeira et al., 2012). The studies on MO have involved application as MO seed powder, MO extract, and purified coagulating proteins from MO seed extract. Nowadays, researchers are continuously working on the extraction and purification of coagulating proteins from the crude MO extract. As per literature, most of the researchers have applied MO seeds and its derivatives for harvesting specific microalgae species from their culture medium. The current work involves harvesting of mixed culture microalgae from pond water to restore water quality as well as obtain useful algae biomass. In the present study, effects of MO extract dose, pH, mixing time, mixing rate, settling time, and initial biomass concentration were evaluated to optimize conditions for cost-effective harvesting of mixed culture microalgae from pond water.

Materials and Methods

Chemicals and coagulant

Sodium chloride (NaCl), hydrochloric acid (HCl), and sodium hydroxide (NaOH) used in the present study were purchased from Loba Chemie, India. All chemicals were of analytical grade. The seeds of *M. oleifera* were harvested in the month of April and May 2017 from the trees located in the campus of NIT Kurukshetra, Haryana. The seeds were sun-dried and kept in an airtight container in the dark till used for the study.

Preparation of MO seed extract coagulant solution

Coagulant from MO seed extract was prepared according to the protocol prescribed by Ndabigengesere, Narasiah, and Talbot (1995) and Barrado-Moreno et al. (2016). Quality seeds were selected, and the shells were removed manually from the pods. Then, pods were sun-dried and grounded to powder by a domestic blender. The powder sieved through 300-μm sieve and retained by 200μm was used in the study. The prepared seed powder was stored in an airtight jar and protected from moisture and light. The MO coagulant solution was prepared by dissolving 5 g of powder in 100 ml of 0.5 M NaCl solution stirred for 30 min using a magnetic stirrer and then filtered through Whatman Filter Paper No 42. Fresh MO extract was prepared for experiments, and the solution was shaken vigorously before use.

The MO extract has total dissolved solids (TDS) concentration 55,680 ± 42.4 mg/L, that is, 1ml MO extract = 55.680 mg of coagulating protein bonded with NaCl. The MO extract solution was used directly as a coagulant so the doses are represented in ml/L and corresponding doses in mg/L are also given in Table 1.

Raw and treated water characterization

Water samples for the present study were collected from a pond at Dayalpur village near NIT Kurukshetra, Haryana, India, in the month of August, 2018. Raw and MO-treated water samples were characterized based on pH, conductivity, total solids, total dissolved solids, total suspended solids, total hardness, chloride, alkalinity, acidity, optical density (OD 532), turbidity, orthophosphate (OP), total phosphorus (TP), total nitrogen (TN), and chlorophyll-a.

Flocculation assay

A laboratory-scale jar test apparatus using 1,000-ml test samples was used to determine flocculation performance under various conditions to study the effect of *M. oleifera* dose, pH, mixing time, mixing rate, settling time, temperature, and initial biomass concentration on microalgae cell, turbidity, chlorophyll-a, and nutrient removal. The jar test apparatus have paddles that can be regulated at the speed range of 30–150 rpm. Flocculation study was conducted at pH values of 3, 5, 6, 7, 8, 9, 10, and 11 and MO dosages of 0, 5, 10, 15, 20, and 25 ml/L. The sample pH was adjusted using 0.1 M HCl or 0.1 M NaOH prior to the flocculation. After adding the required amount of flocculant, samples were rapidly mixed at 150 rpm for 2 min

followed by slow mixing at 30 rpm for 10 min. All samples were given 20-min settling time before carrying out further analysis. Each assay was carried out in triplicate, and average values were calculated. The water samples were analyzed for pH, turbidity, conductivity, total nitrogen, total phosphorus, chlorophyll-a, and optical density (OD 532) before and after treatment using MO as a coagulant. Microalgae cell concentration was measured using optical density (OD 532) (Ahmad et al., 2011; Myers, Curtis, & Curtis, 2013). The microalgae cell removal was calculated using the following equation (Pahl et al., 2013):

$$\text{Microalgae Cell Removal } (\%) = \frac{I_{\text{blank}} - I_{\text{sample}}}{I_{\text{blank}}} \times 100.$$

where I_{blank} is the optical density (532 nm) of the raw pond water (control) with no coagulant and I_{sample} is the optical density (532 nm) of the sample after the coagulation process.

Statistical analysis

All the flocculation experiments were conducted in triplicates, and the means of the removal efficiencies were analyzed using one-way ANOVA. The significance level was established at $p = .05$ unless otherwise noted. The values are expressed as "Average value ± *SD*," and the standard deviations are shown by error bars for removal efficiency.

Parameters analyzed

The pH, turbidity, conductivity, total nitrogen (TN), total phosphorus (TP), chlorophyll-a, and optical density (OD 532) of the raw and treated water samples were determined as per Standard Methods (APHA, 2005). The pH value and conductivity were measured using a Thermo Scientific Orion 5-Star Benchtop Analyser. Turbidity was measured with Hach Turbidity Meter (2100AN), and the optical density was measured using a UVS 2700 Labomed Spectrophotometer. MO Seed powder was characterized by Fourier transform infrared spectroscopy (FTIR) using SHIMADZU Infrared Spectrophotometer. The spectra of FTIR were obtained in the range from 4,500 to 400 cm^{-1}.

Results and Discussion

FTIR study for characterization of MO

Ndabigengesere et al. (1995) mentioned that the coagulation property of MO had been already proved but the mechanism of the reaction is very complex and not fully explained. They characterized the coagulating component as water-soluble material, which on purification resembled to be the proteins. Similarly, Gassenschmidt, Jany, and Tauscher (1991), Gassenschmidt et al. (1995) reported the active coagulating material to be protein. Ghebremichael et al. (2005) reported that the salt extract gives better results because of higher protein content compared to water extract. Also, this coagulating protein is thermoresistant and remains active after 5-hr heat treatment at 95°C. Their characterization signifies that the coagulating protein is not a single, homogenous protein but it is the mixture of proteins with similar physical characteristics. On the other hand, Okuda, Baes, Nishijima, and Okada (2001) reported that the active coagulating compound was neither protein, polysaccharide, nor lipids. They characterized it as an organic polyelectrolyte with a molecular weight of Ca. 3,000. The IR of the purified active component was the amino group, hydroxide group, and little amount of carboxyl group. Behera and Balasubramanian (2019) reported that along with protein, carbohydrate present in MO extract also aids in the coagulation process.

In the present study, FTIR analysis was done from 400 to 4,500 cm^{-1} to identify the presence of active functional groups in MO that assist in the flocculation of microalgae (Figure 1). The IR spectra show strong broad bands in the region of 3200–3500 cm^{-1} with maximum absorbance at 3,350 cm^{-1}. The FTIR spectra show other strong sharp bands at 2,924 and 2,852 cm^{-1}, three strong absorption bands at 1,747, 1,652, and 1,541 cm^{-1}, and absorption bands with medium intensity at 1,458, 1,240, and 1,056 cm^{-1}. The large bands in the region of 3,200–3,500 cm^{-1} may be attributed to the presence of hydroxyl groups (OH) that might be present in proteins, fatty acids, carbohydrates, and lignin (Baptista et al., 2017). The bands present at 2,923 and 2,852 cm^{-1} signify the C–H–CH_2 group that is present in fatty acids (Reck et al., 2019). The two strong absorption bands at 1,656 and 1,542 cm^{-1} usually signify amide I and II, respectively, which are in the usual range of absorption of helices of proteins, confirming the presence of proteins in the coagulating active materials (Kwaambwa & Maikokera, 2008). The FTIR results of the present study are in conjunction with the study by Baptista et al. (2017) and Reck et al. (2019). They fractionalized the coagulating protein of MO seeds into albumin and globulin for conducting coagulation. The IR spectra of the present study and IR spectra of protein fractions reported earlier are similar and confirm the presence of proteins as active coagulating agents in the MO extract. The solubility of proteins usually increases in the presence of salts while using the salting-in mechanism for preparing an extract from MO seed powder.

Effect of MO extract addition on water characteristics

To assess the effect of MO extract addition on pH, conductivity, turbidity, orthophosphate, TP, and TN of water, varying doses of MO extract solution ranging from 0 to 25 ml/L were added in 1,000 ml distilled water (DW) samples. The variations in pH, conductivity, turbidity, orthophosphate, TP, and TN of water due to varying dose of MO extract addition in the water are given in Table 1. The addition of MO extract shows a slight increase in pH of the distilled water from 4.71 to 5.03, whereas the conductivity increased from 2.21 to 307 μS/cm for an increase in dose from 0 to 5 ml/L. With a further increase in dose of MO extract from 5 to 25 ml/L, the pH of distilled water was increased slightly from 5.03 to 5.40. However, for the same increase in dose, conductivity increased from 307 to 1,376 μS/cm. MO extract contains cationic water-soluble proteins in which alkaline amino accepts protons with release of hydroxyl groups making the water more alkaline and results in an increase of pH of distilled water (Hendrawati et al., 2016). Earlier, Hendrawati et al. (2016) observed a slight increase in pH

from 7.60 to 8.28 of the distilled water on the addition of MO extract. On the other hand, some researchers have concluded that MO extract does not alter the pH and conductivity of the solution (Ndabigengesere & Narasiah, 1998; Ndabigengesere et al., 1995). In the present study, an increase in pH and conductivity was observed due to the presence of NaCl in the MO extract whereas in the previous studies either purified extracted protein of MO or tap water was used for preparing extract solution. The purification of the coagulating protein can be carried by various methods such as dialysis, ultrafiltration, lyophilization, and ion exchange but these methods are not economical for harvesting microalgae from surface water (Ndabigengesere & Narasiah, 1998). Also, the studies by Okuda, Baes, Nishijima, and Okada (1999), Madrona et al. (2012), and Ghebremichael et al. (2005) concluded that salt solution is most effective in the extraction of proteins compared to the deionized water. The aim of the present study was to harvest microalgae in an economical way; thus, the purification of the extracted protein was not carried out. Researchers have characterized MO extract and reported the presence of large numbers of ions such as Ca^{2+}, NO_3^-, and PO_4^{-3}, (Ndabigengesere & Narasiah, 1998; Sánchez-Martín, Beltrán-Heredia, & Peres, 2012). Table 2 shows the characteristics of MO seeds extract solution used in the present study vis-a-via characteristics of extract reported by Ndabigengesere and Narasiah (1998) and Sánchez-Martín et al. (2012). The MO extract is light brown in color with a turbidity of 18.35 ± 0.07 NTU but when the MO extract is added to distilled water, it turns the distilled water to milky white. The salt extract has high ionic strength, and due to salts, it results in protein–protein dissociation or protein–protein solubility. However, when the salt extract is added to distilled water the reduced ionic strength results in salting-out process which causes protein–protein association or decrease in protein solubility. This insoluble protein gives milky white appearance to the solution. Table 1 shows the increase in turbidity of distilled water with an increase in dose of MO extract due to the increase in milky white appearance in the solution. The increase in turbidity is due to the presence of insoluble proteins of MO extract which increases with the increasing dose of MO extract. Turbidity increased from 0.405 to 528 NTU with the increase in MO extract dose from 0 to 25 ml/L. The EC also increases with the increasing dose of MO extract due to higher salt contribution by MO extract. Since MO extract solution contains 106.50 ± 10.60 mg/L TP and 1,394.50 ± 23.33 mg/L TN, its addition to distilled water increases concentration of the nutrients in distilled water sample. The increase in dose of MO extract from 0 to 25 ml/L increased the TN from 0 to 37.60 mg/L, TP from 0 to 2.98 mg/L, and orthophosphate concentration from 0 to 0.23 mg/L. The increase in the concentration of nutrients was found to be proportional to the dose of MO extract added.

Effects of varying MO extract dose on microalgae cell, turbidity, chlorophyll-a, and nutrient removal

The effect of varying dose of MO extract was studied by conducting the jar test at pH 8.88, MO dosages of 0, 5, 10, 15, 20, and 25 ml/L, rapid mixing at 150 rpm for 2 min followed by slow mixing at 30 rpm for 10 min, and 20-min settling time. All tests were performed at room temperature. The Figure 2 shows the effects of varying MO extract dose on the removal of microalgae cells, turbidity, chlorophyll-a, and nutrients. The removal of microalgal cells was increased from 90.08% to 94.35% with an increase in dose from 5 to 15 ml/L. Similarly, turbidity removal increased from 94.13% to 97.63% for the same MO extract dose. However, there was a slight decrease in microalgae cell removal with a further increase in MO extract dose. Baharuddin et al. (2016) harvested *N. oculata* using MO extract and observed maximum removal of 70.56% at a dose of 4,000 mg/L. However, an increase in coagulant dose to 5,000 mg/L reduced microalgae cell removal to approximately

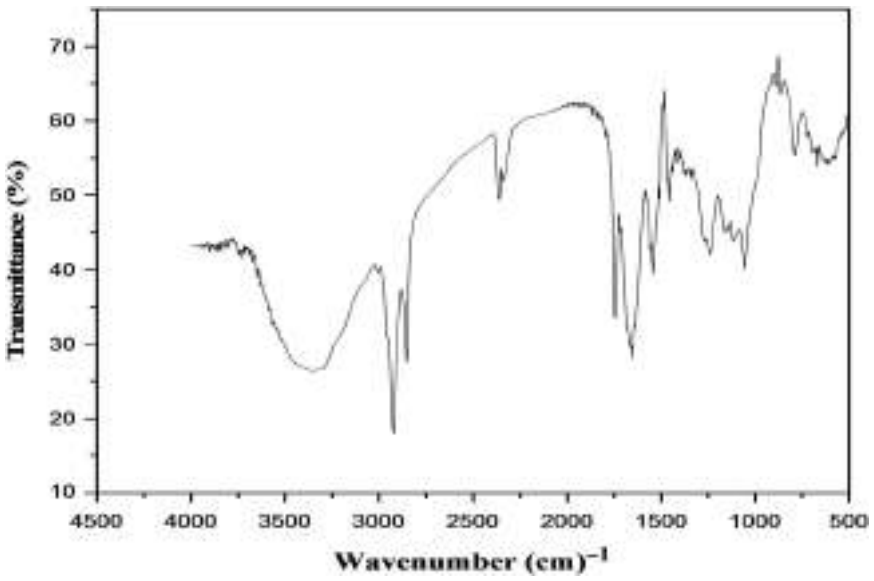

Figure 1. FTIR spectra of MO seed powder.

Table 2. Characteristics of MO seed extract coagulant solution

S.NO.	PARAMETERS	CHARACTERISTICS AS PER PRESENT STUDY	CHARACTERISTICS AS PER NDABIGENGESERE AND NARASIAH (1998)	CHARACTERISTICS AS PER SÁNCHEZ-MARTÍN ET AL. (2012)
1.	pH	5.28 ± 0.014	5.8	NA
2.	Conductivity (mS/cm)	44.60 ± 0.28	1.7	NA
3.	Total dissolved solids (mg/L)	55,680 ± 42.4	NA	NA
4.	Turbidity (NTU)	18.35 ± 0.07	NA	NA
5.	Total phosphorous (mg/L)	106.50 ± 10.60	NA	70
6.	Total nitrogen (mg/L)	1,394.50 ± 23.33	942[a]	610[b]
7.	Orthophosphate (mg/L)	37.44 ± 2.25	187	50
8.	Nitrate (mg/L)	211.89 ± 20.07	140	550

Abbreviation: NA, not available.

[a]TKN + nitrate.

[b]Ammonia + nitrate.

50%. The microalgae removal with varying doses observed in the present study and reported by Baharuddin et al. (2016) has a similar trend but Baharuddin et al. (2016) achieved relatively lower microalgae removal for MO extract prepared using MO seeds without oil extraction. The reasons for varied microalgae removal efficiencies could be different cell surface properties of mixed microalgae in the present study and specific microalgae species *N. oculata* in the study by Baharuddin et al. (2016), varied culture conditions, and growth phase of the microalgae. Chlorophyll-a removal was 89.99 at 5 ml/L dose, and it increased to 98.30% with an increase in dose from 5 to 25 ml/L. The 15 ml/L dose results in 97.39% chlorophyll-a removal and considered optimum dose. Ndabigengesere et al. (1995) observed an increase in residual turbidity with the increase in coagulant dose beyond the optimal dose and explained that re-distribution caused by the reversal of colloidal charge due to excess of adsorption results in increased residual turbidity. Microalgae cells, turbidity, and chlorophyll-a concentration are interrelated; thus, the trends for their removal in the present study were similar to each other. Barrado-Moreno et al. (2016) successfully removed 90% chlorophyll-a at 25 mg/L dose of MO extract and observed that higher than 25 ml/L dose did not contribute more chlorophyll-a removal. MO extract characterization shows the presence of nitrogen and phosphorous in the solution. The maximum removal of TN and TP was 48.08% and 56.76% at dose of 10 and 5 ml/L, respectively. The further increase in dose contributed more N and P addition by MO extract, and it resulted in lower TN and TP removal and even increase in TN beyond 20 ml/L dose rather than removal. At optimum conditions, TN and TP removal were 26.70% and 36.93%, respectively.

Effects of pH on microalgae cell, turbidity, chlorophyll-a, and nutrient removal

The coagulation/flocculation process is highly pH-dependent. The effect of varying pH was studied by conducting the jar test at pH values of 3, 5, 6, 7, 8, 9, 10, and 11, optimum dose of 15 ml/L, rapid mixing at 150 rpm for 2 min, followed by slow mixing at 30 rpm for 10 min, and 20 min settling time. All tests were performed at room temperature. The Figure 3 shows the effects of variations in pH on microalgae cell, turbidity, chlorophyll-a, and nutrient removal. The removal efficiency of microalgae cells increases with increase in pH from 3 to 8; after pH 8, the removal efficiency reduces. The pond water samples at pH 3 and 11 show autoflocculation when left for some time before the jar test. The microalgae cells in sample float at the water surface at pH 3 while the microalgae cells flocculate to the bottom of the jar at pH 11. The autoflocculation of microalgae at pH 3 is due to the concentration of H^+ ions that destabilize by protonation of negatively charged microalgal cells, whereas autoflocculation at pH 11 is due to the precipitation of metal salts (Sánchez-Martín et al., 2012). When the pH increased from 3 to 8, the microalgae cell removal efficiency increased from 86.95% to 96.33% and then a reduction of 3.92% was observed in removal efficiency with further increase in pH to 11. Baharuddin et al. (2016) also observed a reduction in microalgae removal efficiency with an increase in pH from 8 to 11. Baharuddin et al. (2016) flocculated *N. oculata* using an extract of MO seed powder produced after oil extraction (MOAE) and without oil extraction (MOWE) and observed maximum removal of microalgae cell at pH 7 with a removal efficiency of 93.77% and 70.56%, respectively. Below pH 7, there was a slight reduction in microalgae cell removal. Knuckey et al. (2006) harvested *N. oculata* and *Isochrysis* sp. with pH adjustment and observed >30% removal efficiency of microalgae cell at higher pH. Teixeira et al. (2012) observed 89% of microalgae cell removal at pH 9.2 with 1,000 mg/L dose of MO. The microalgae cell, turbidity, and chlorophyll-a removal show similar trend with maximum removal efficiency of 96.33%, 97.39%, and 97.71%, respectively, at pH 8. At low pH of 3 and 5, MO extract addition increased the TP concentration from 2.05 ± 0.10 to 3.62 ± 0.14 and 2.05 ± 0.10 to 3.36 ± 0.05 mg/L, respectively, and no TP removal was observed. This increase in TP is because of no role of MO extract in flocculation and removal of microalgae cells results primarily due to autoflocculation. Diamadopoulos and Benedek (1984) have

studied precipitation of phosphorous through pH variations in the presence and absence of coagulants. They concluded that phosphorus is released or its solubility is increased at low pH (<6), whereas at high pH (>6), phosphorous is precipitated with calcium. The TP removal efficiency increased with a further increase in pH, and it was maximum at pH 11. At pH 11, autoflocculation results due to precipitation of microalgae cells and metal salts which might have entrapped coagulant also and resulted in high TP removal. The TN removal at pH 3 was only 20.85% but it increased with an increase in pH and was found to be maximum, that is, 49.17% at pH 10. The low TN removal at pH 3 and 5 is mainly due to the removal of microalgae cells floated at the surface of the water. The aggregation of microalgae cells is due to autoflocculation. At pH 3 and 5, the MO extract does not actively participate in flocculation. Above pH 5, the removal of TN increases due to the active participation of MO extract for coagulating the microalgae cells. The NO_3^- ions present in water have to compete with other anions like phosphate or chloride ions for adsorption by MO extract proteins, and due to this, low nitrate removal causes slight variation in TN removal at pH 6, 7, and 8. Bhatnagar and Sillanpää (2011) reported that at pH less than 9, other anions reduced the NO_3^- adsorption in the order of carbonate > phosphate > chloride > sulfate due to competition with nitrate for removal by adsorption. The removal of TN increases with an increase in pH above 9 because at higher pH metal hydroxides entrap coagulated microalgae cells as well as MO extract proteins. The TN removal was only 25.48% at optimum pH 8. The raw water TN comprises of TKN and NO_3^- N, and the MO extract used as coagulant also contains higher TN with 15.19% nitrate nitrogen (Table 2). The low TN removal is due to the dissolved nitrogen content present in pond water and residual dissolved nitrogen including nitrate nitrogen contribution by the MO extract. The removal of suspended microalgae cells, other suspended organic nitrogen content, and actively involved MO coagulating proteins mainly contributed in observed low TN removal. Aguilar, Saez, Llorens, Soler, and Ortuno (2002) observed that ammonia nitrogen removal is not directly related to coagulation–flocculation process and reported that removal of ammonia using MO seed powder did not give good results because MO is a source of cationic coagulants. Ammonium ions cannot be removed due to the positive charges of both MO coagulating proteins and ammonium ions. Zaid and Ghazali (2019) reported that removal of nitrate is probably due to the attraction between the positive charge of MO seed powder and the negatively charged nitrate ions. However, if the dosage of MO seed powder used is too high, it increases substantial nitrate content in water (Ghebremichael et al., 2005; Ndabigengesere & Narasiah, 1998).

Effects of mixing time on microalgae cell, turbidity, chlorophyll-a, and nutrient removal

When MO extract is rapidly mixed with pond water, the cationic protein from MO destabilizes the microalgae cells and forces them to bind together to form a precipitate. So, sufficient mixing is required for the interaction of negatively charged microalgae cells with the cationic protein present in MO extract. The effect of varying mixing time was studied by conducting the jar test at pH 8, dose 15 ml/L, rapid mixing at 150 rpm for 2 min followed by slow mixing at 30 rpm for the varying mixing time of 2, 5, 7, 10, 12, and 15 min, and 20-min settling time. All tests were performed at room temperature. Figure 4 shows the effects of mixing time on microalgae cell, turbidity, chlorophyll-a, TP, and TN removal. In the present

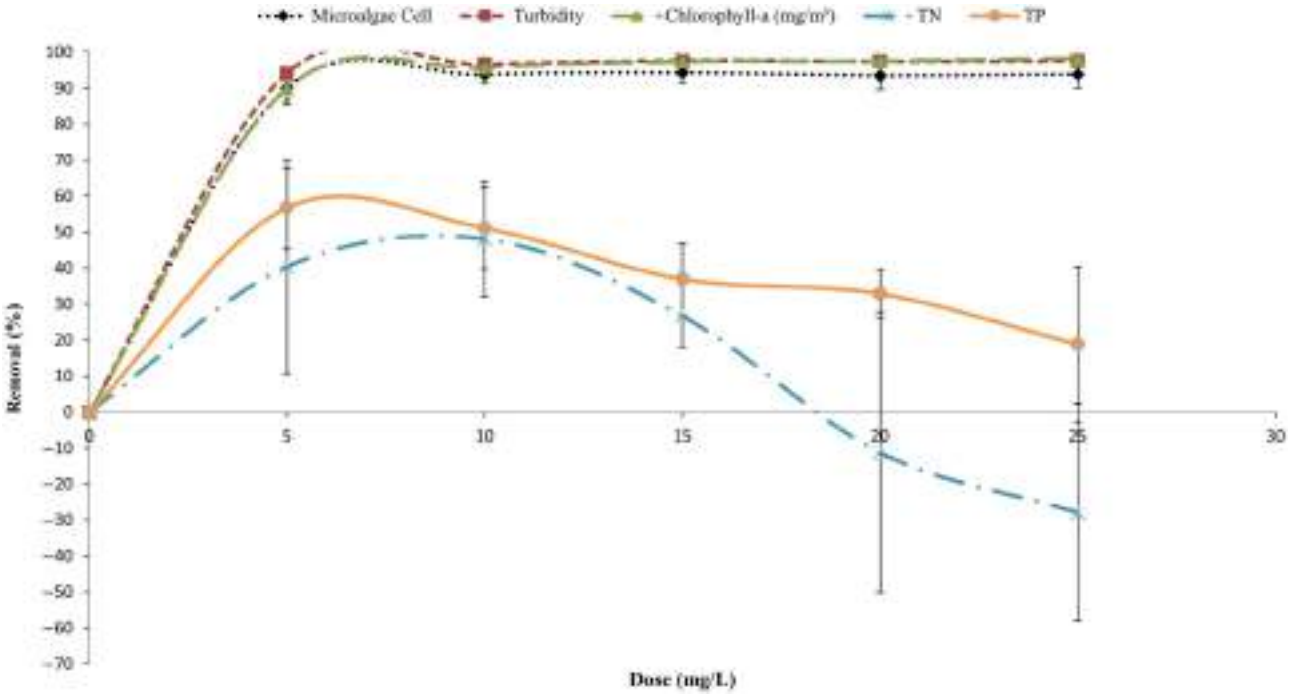

Figure 2. Effects of varying doses of MO extract on microalgae cell, turbidity, chlorophyll-a, and nutrient removal.

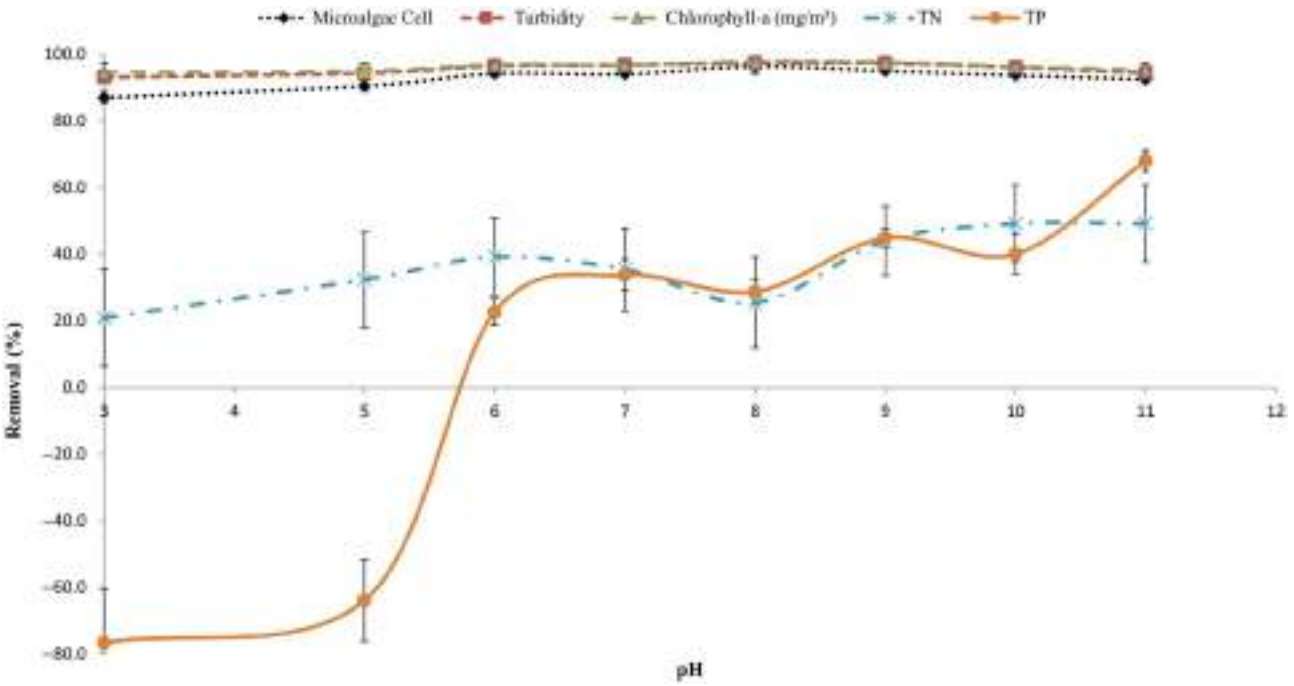

Figure 3. Effects of variation in pH on microalgae cell, turbidity, chlorophyll-a, and nutrient removal.

study, variation in mixing time from 2 to 15 min did not show a significant effect on the removal efficiency of microalgae cells, turbidity, chlorophyll-a, and TP. The TN removal was only 5.68% for 2 min mixing time. With a further increase in mixing time to 3 min, TP removal increased from 5.68% to 24.66%. For mixing times ranging from 2 to 15 min, microalgae cell, turbidity, and chlorophyll-a removal were >90% and TP removal was 31%–33%. For further study, 5-min mixing time was considered to be optimum mixing time to help the binding of cationic protein with the negatively charged microalgae cells. The increase in mixing time above 5 min even resulted in the reduction of TN removal. Barrado-Moreno et al. (2016) observed maximum efficiency for chlorophyll-a removal for 30-min mixing time.

Effects of mixing rate on microalgae cell, turbidity, chlorophyll-a, and nutrient removal

Flocs formed by MO extract are highly fragile which breaks with sudden movements and results in loss of effectiveness (Sánchez-Martín et al., 2012). Ndabigengesere and Narasiah (1996) suggested that soft and continuous stirring enhances the formation and compaction of MO extract flocs. The effect of varying mixing rate was studied by conducting the jar test at pH 8, dose 15 ml/L, rapid mixing at 150 rpm for 2 min followed by varying mixing at 30, 75, and 150 rpm for 5 min of mixing time, and 20-min settling time. All tests were performed at room temperature. Figure 5 shows the effects of mixing rate on microalgae cell, turbidity, chlorophyll-a, and nutrient removal. The increase in mixing rate from 30 to 150 rpm did not alter the removal efficiency and remained about 92%, 97%, and 97% for microalgae cells, turbidity, and chlorophyll-a, respectively. The removal efficiency of TP increased from 21.21% to 39.85% with an increase in mixing rate from 30 to 150 rpm, whereas TN removal decreased from 39.24% to 24.53% with an increase in the mixing rate. A slight loss of removal efficiency of microalgae cells, turbidity, and chlorophyll-a was observed at higher mixing rates. A similar loss of removal efficiencies due to the breakage of the flocs at higher mixing rates was observed by Sánchez-Martín et al. (2012).

Effects of settling time on microalgae cell, turbidity, chlorophyll-a, and nutrient removal

The settling time depends on the floc size (Ahmad, Sumathi, & Hameed, 2006). The effect of settling time was studied by conducting the jar test at pH 8, dose 15 ml/L, rapid mixing at 150 rpm for 2 min followed by slow mixing at 30 rpm for 5 min of mixing time, and varying settling time of 5, 10, 15, 20, 25, and 30 min. All tests were performed at room temperature. Figure 6 shows the effects of settling time on microalgae cell, turbidity, chlorophyll-a, and nutrient removal by MO extract. The increase in settling time from 5 to 30 min resulted in an increase in removal efficiencies of microalgae cells, turbidity, chlorophyll-a, and nutrients. From an initial 5- to 20-min increase in settling time, removal efficiencies of microalgae cells, turbidity, and chlorophyll-a increased from 77.70% to 94.57%, 78.11% to 96.20%, and 79.47% to 96.49%, respectively. Then, with further increase in settling time up to 30 min, there was no significant increase in the removal of microalgae cells, turbidity, chlorophyll-a, and nutrients. For the initial 5-min settling time, TP concentration increased from 2.05 ± 0.10 to 2.65 ± 0.15 mg/L and TN concentration increased from 20.37 ± 4.64 to 20.86 ± 1.30 mg/L. The increase in TP and TN might be because of poor microalgae cell removal efficiency of only 77.70% due to inadequate settling and contribution

of N and P by MO extract. However, with an increase in settling time TP removal efficiency increased correspondingly. At 20-min settling time, microalgae cell, turbidity, chlorophyll-a, TN, and TP removal efficiency were 94.57%, 96.20%, 96.49%, 21.92%, and 23.90%, respectively, and it was considered optimum settling time for further study.

Effects of temperature on microalgae cell, turbidity, chlorophyll-a, and nutrient removal

To assess effects of temperature on flocculation of mixed culture of microalgae, the temperature varied between 10 and 45°C, while other optimized experimental conditions were dose 15 ml/L of MO extract, pH 8, rapid mixing at 150 rpm for 2 min, slow mixing at 30 rpm for 5 min, and 20-min settling time. Low temperatures were achieved by storing water in a refrigerator before use. Intermediate temperatures were achieved by mixing refrigerated and control water. The higher temperatures were achieved by keeping the jar test beaker on a hot plate. The very low temperature was maintained during experiments by placing the jar test beaker in an insulated vessel filled with iced water. The temperatures were controlled to ±3°C in the range of 10–45°C during the present study. Figure 7 shows the effects of varying temperatures (10, 25, 35, 45°C) on microalgae cell, turbidity, chlorophyll-a, and nutrient removal. The variation in temperature did not affect the removal of microalgae cells and turbidity. However, increasing temperature from 12 to 25°C caused a slight increase in chlorophyll-a removal, but thereafter it remained almost the same. The TN removal did not show significant variation with temperature, and removal was maximum at 25°C. The TP removal was maximum at 12°C. The results show that temperature variation did not affect the coagulation of microalgae significantly, and therefore, the performance of the coagulating process is not likely to be affected due to seasonal temperature changes. Ndabigengesere and Narasiah (1996) studied the effect of temperature on turbidity removal by MO seeds extract and reported a slight decrease in turbidity removal efficiency with a reduction in temperature from 25 to 10°C. The decrease in turbidity removal is due to the change in viscosity and density of water with temperature. Othman, Bhatia, and Ahmad (2011) reported reduced efficiency of the *M. oleifera* coagulation when temperature of the wastewater was increased from 30 to 70°C. The low strength flocs formed at high temperature are easily broken and decrease the suspended solid removal efficiency. The turbidity removal is usually maximum at room temperature, that is, 22–27°C, and too high or too low temperatures show slight variations in turbidity removal efficiencies (Ndabigengesere & Narasiah, 1996; Othman et al., 2011). The results of the present study and studies by Ndabigengesere and Narasiah (1996) and Othman et al. (2011) show negligible variations in turbidity removal between temperature variations 10 to 45°C.

Effects of biomass concentration on microalgae cell, turbidity, chlorophyll-a, and nutrient removal

To assess the effects of biomass concentration, a varying quantity of distilled water was added to pond water to obtain a mixture of pond water with varying biomass concentration. The mixture of distilled water and pond water represents varying concentrations of biomass in pond water in various seasons of the year. Table 3 shows the quantity of distilled water added to pond water to obtain desired dilutions and the corresponding initial biomass concentration. The effects of varying algal biomass concentrations were

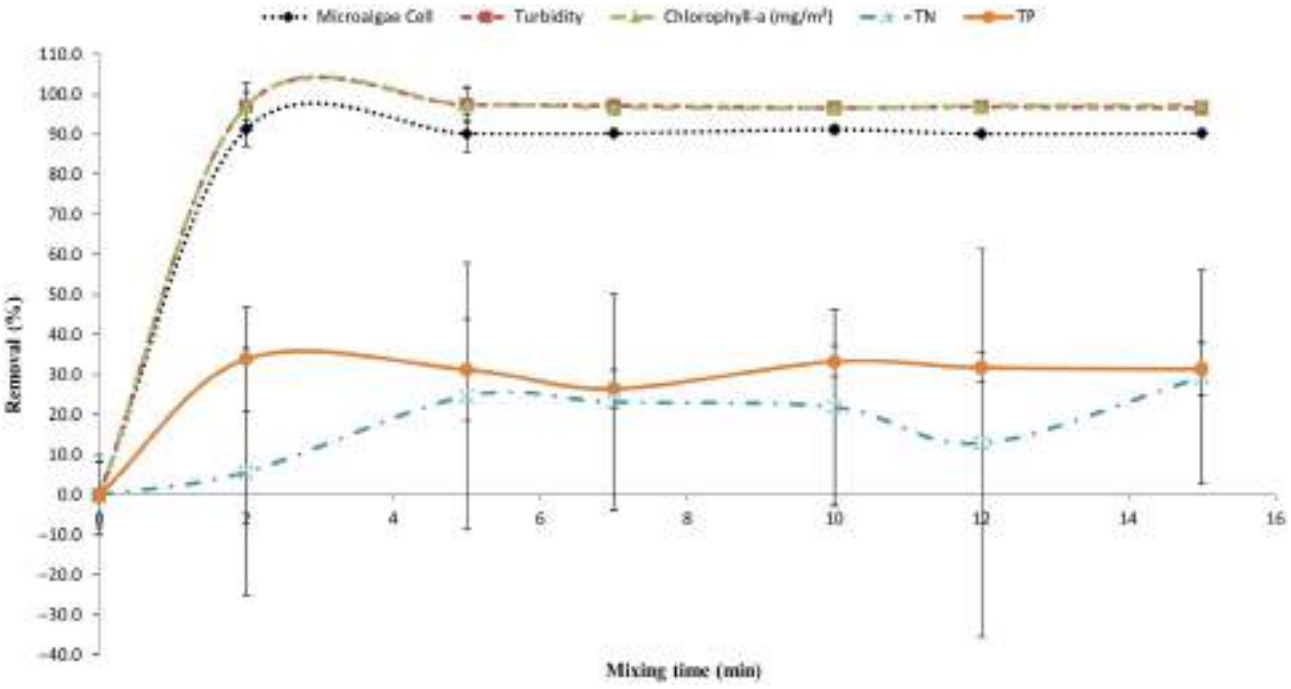

Figure 4. Effects of variation in mixing time on microalgae cell, turbidity, chlorophyll-a, and nutrient removal.

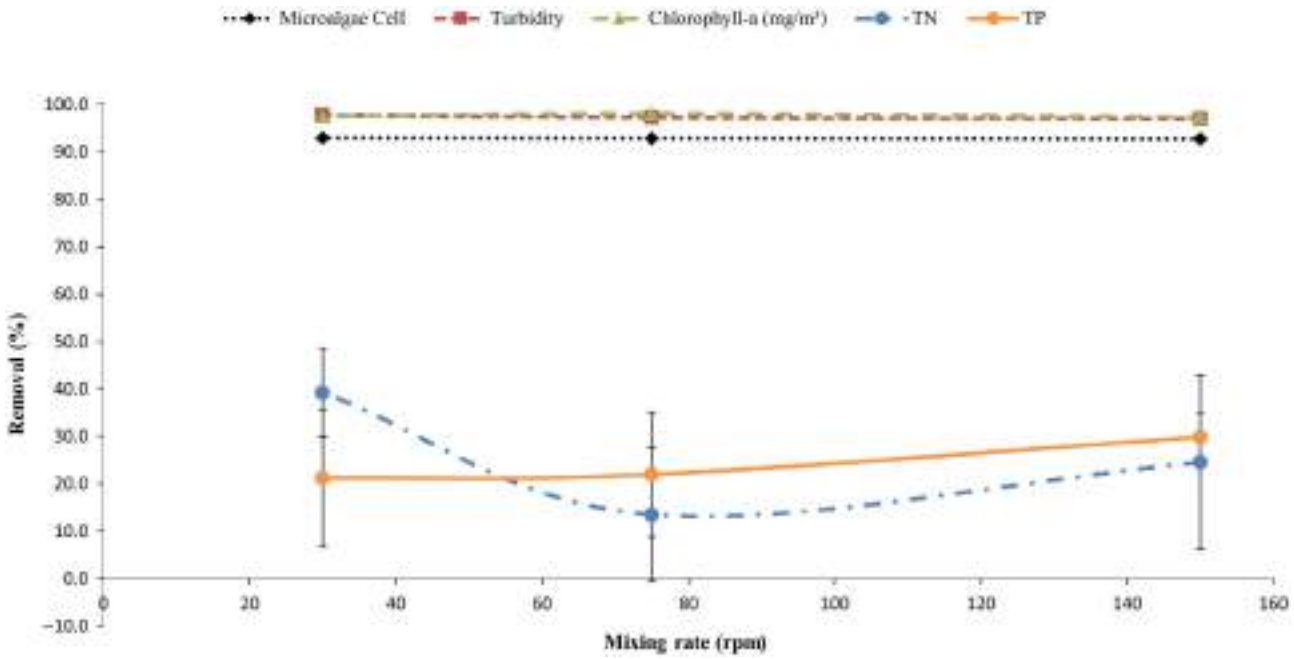

Figure 5. Effects of variation in mixing rate on microalgae cell, turbidity, chlorophyll-a, and nutrient removal.

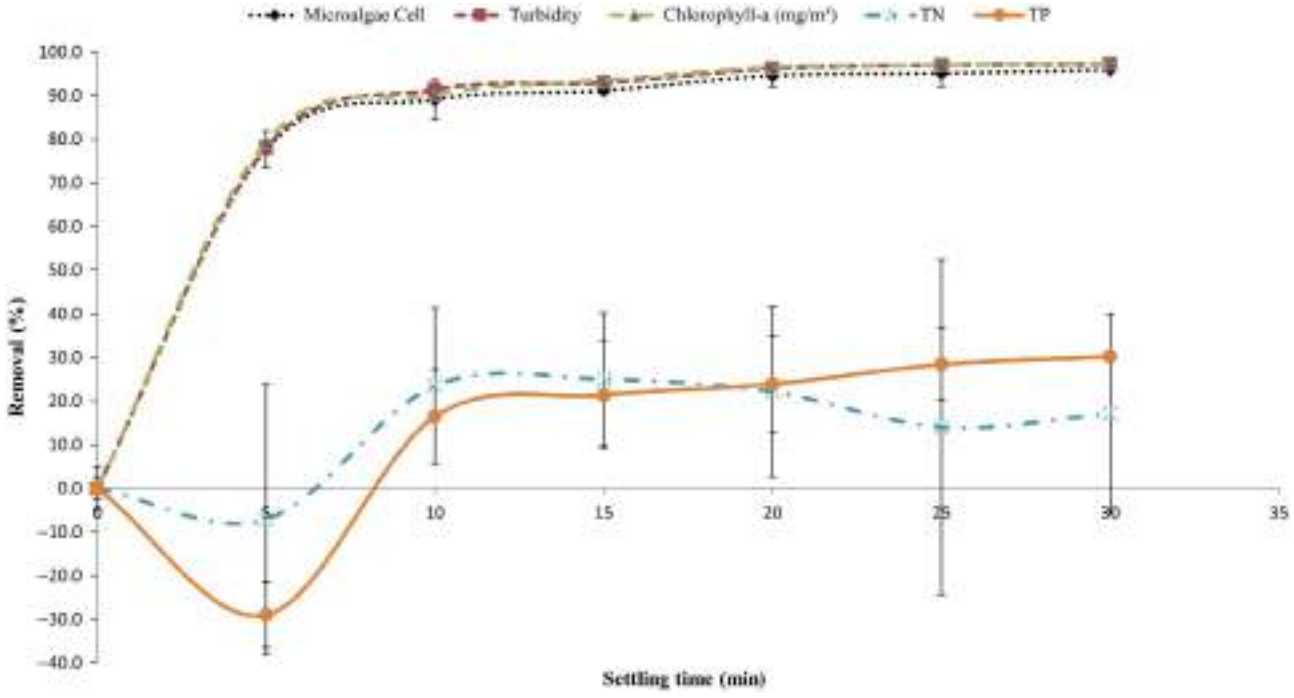

Figure 6. Effects of variation in settling time on microalgae cell, turbidity, chlorophyll-a, and nutrient removal.

assessed by performing jar test experiments at optimum conditions, that is, 15 ml/L of MO extract dose; pH 9; mixing time 5 min; mixing rate 30 rpm; and settling time 20 min. The control was pond water without any coagulant addition. Figure 8 shows the effects of varying biomass concentration on microalgae cell, chlorophyll-a, turbidity, TP, and TN removal. The 100% mixture of pond water with initial biomass concentration 181.413 mg/L shows the optimum result for harvesting mixed culture of microalgae with microalgae cell, chlorophyll-a, and turbidity removal 92.97%, 97.82%, and 97.62%, respectively. However, TP removal was minimum in undiluted pond water with a biomass concentration of 181.41 mg/L. The TN concentration increases because of the addition of MO extract, and its removal was

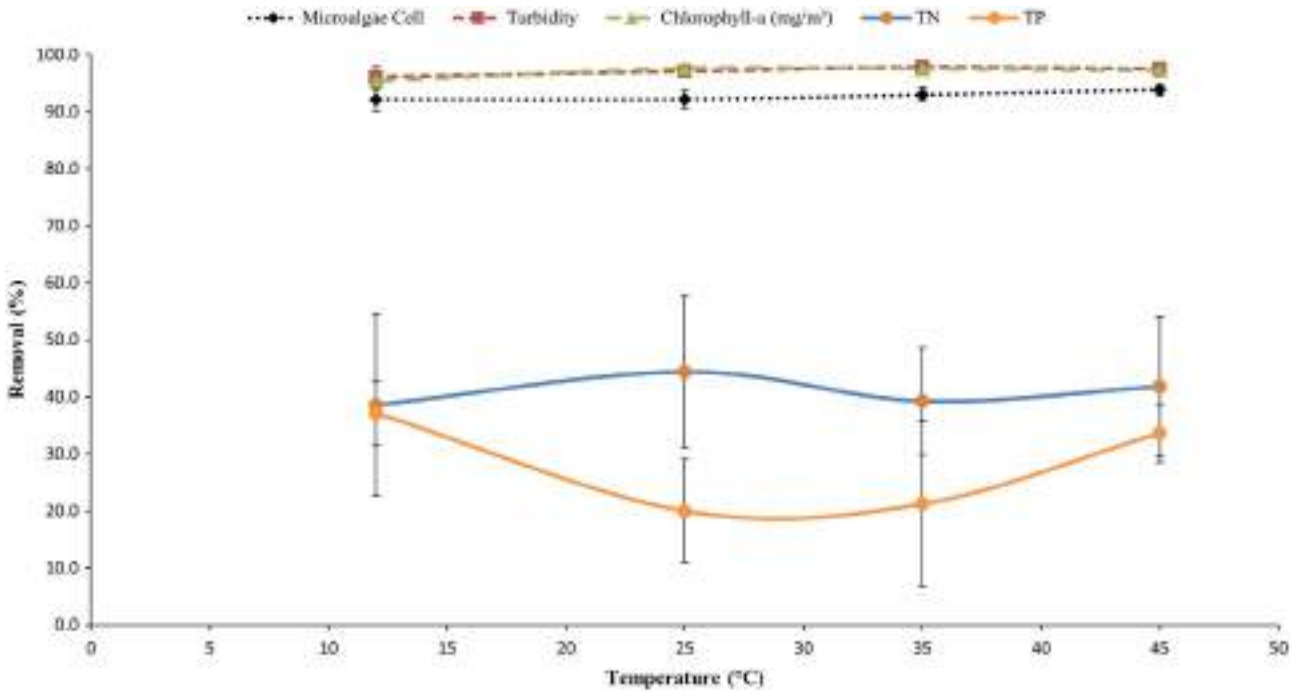

Figure 7. Effects of variation in temperature on microalgae cell, turbidity, chlorophyll-a, and nutrient removal.

Table 3. Biomass concentration in pond water mixtures at various dilutions with distilled water

S.NO.	MIXTURE OF POND WATER (%)	SAMPLE		BIOMASS CONCENTRATION (MG/L)
		POND WATER (ML)	DISTILLED WATER (ML)	
1	25	250	750	37.438
2	50	500	500	91.429
3	75	750	250	136.421
4	Undiluted Water	1,000	0	181.414

found to be maximum in undiluted pond water with a biomass concentration of 181.41 mg/L. The microalgae cell removal efficiency was minimum in 25% mixture of pond water with an initial biomass concentration of 91.42 mg/L. The chlorophyll-a removal efficiency and turbidity removal efficiency were minimum in 75% mixture of pond water with initial biomass concentration 136.42 mg/L. The results show that if there is any fluctuation in the pond water algal biomass concentration, it will not significantly affect microalgae cell, turbidity, and chlorophyll-a removal. But at a low concentration of microalgae biomass, the removal efficiency of nutrients was low due to the addition of extra nutrients from the MO extract which was not actively participated in the coagulation of microalgae cells.

Effect of microalgae harvesting using MO extract on water quality improvement

The effect of microalgae harvesting using MO extract on water quality improvement was assessed based on the physiochemical characterization of raw and treated water. The important physicochemical characteristics of raw and treated water using MO extract at optimum conditions are summarized in Table 4. Harvesting microalgae from the pond water by using MO extract as coagulant shows reduction in pH, total suspended solids, total hardness, alkalinity, acidity, microalgae cell, turbidity, chlorophyll-a, and nutrient concentration. The pH value decreased from 8.88 ± 0.10 to 7.41 ± 0.45 which is within the acceptable limit for drinking water. Earlier, Ndabigengesere et al. (1995) and Ndabigengesere and Narasiah (1998) reported that the addition of MO extract did not alter the pH and conductivity of water but in present study slight decrease in pH and significant increase in conductivity were observed because of the presence of NaCl binded with MO proteins, whereas Ndabigengesere et al. (1995) and Ndabigengesere and Narasiah (1998) had purified the MO extract. Oluduro and Aderiye (2007a) reported a change in pH due to precipitation of insoluble products of the reaction between MO seeds and hardness causing ions present in water. Conductivity and total solid concentration increased from 869.33 ± 15.14 to 1,631.33 ± 7.02 μS/cm and 941.33 ± 31.13 to 1,410 ± 20.88 mg/L, respectively. The increase in conductivity and total solids is because of an increase in total dissolved solids due to the addition of MO extract as a coagulant. The high total dissolved solids

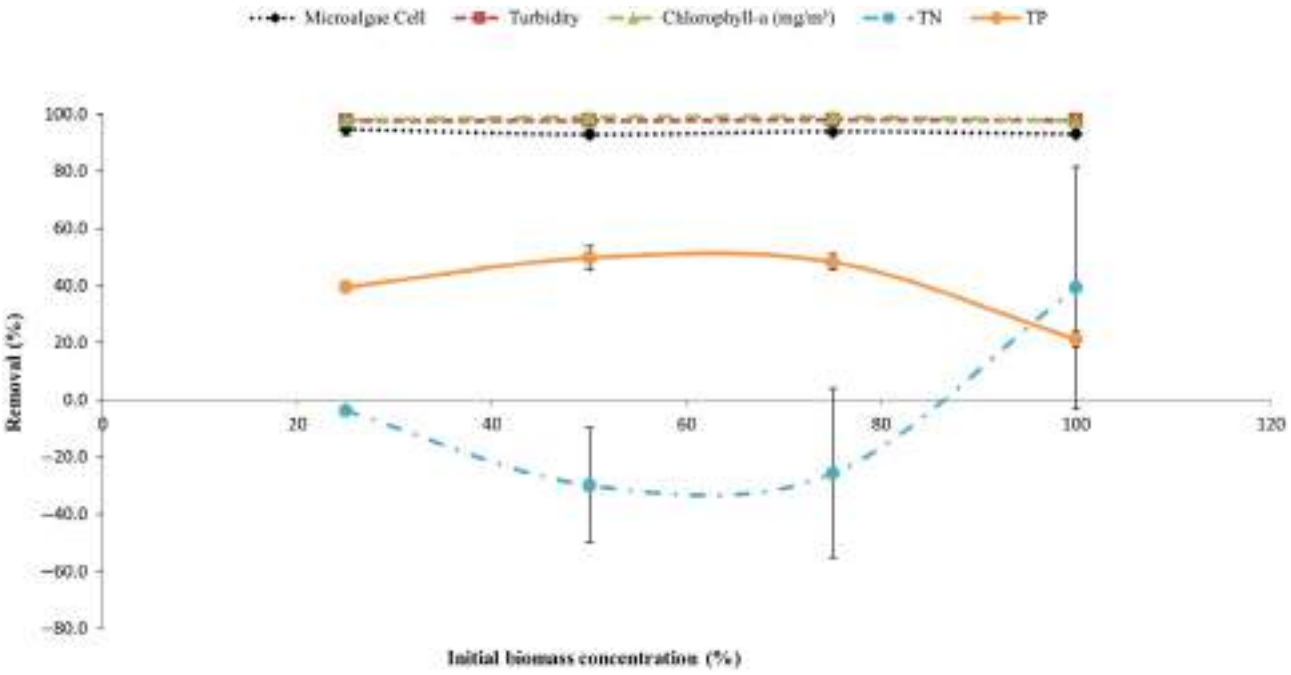

Figure 8. Effects of variation in biomass concentration on microalgae cell, turbidity, chlorophyll-a, and nutrient removal.

Table 4. Characteristic of raw and treated water

S.NO.	PARAMETERS	RAW WATER QUALITY	TREATED WATER QUALITY[A] (AT DOSE 15 ML/L) VALUE	REMOVAL %	TREATED WATER QUALITY[A] (AT DOSE 10 ML/L) VALUE	REMOVAL %
1	Temperature (°C)	32 ± 1.0	34.67 ± 0.58	−8.33[b]	33.45 ± 0.28	−4.53[b]
2	pH	8.88 ± 0.10	7.41 ± 0.45	16.59[b]	7.82 ± 0.36	11.97
3	Conductivity (μS/cm)	869.33 ± 15.14	1,631.33 ± 7.02	−87.65[b]	1,358.23 ± 5.11	−56.24[b]
4	Total solids (mg/L)	941.33 ± 31.13	1,310 ± 20.88	−49.79[b]	1,092 ± 26.8	−16.01[b]
5	Total dissolved solids (mg/L)	629.66 ± 6.65	1,260 ± 24.58	−100.11[b]	1,048 ± 9.0	−66.44[b]
6	Total suspended solids (mg/L)	311.66 ± 34.26	50 ± 4.0	51.87	44 ± 8.0	85.88
7	Total hardness (mg/L as $CaCO_3$)	178 ± 2.0	148 ± 2.0	16.85	145 ± 1.1	18.54
8	Chloride (Cl-) (mg/L)	62 ± 1.15	292 ± 5.29	−288.24[b]	190 ± 3.8	−204.81[b]
9	Alkalinity (mg/L as $CaCO_3$)	266.66 ± 1.15	236 ± 4.0	11.50	215 ± 3.0	19.38
10	Acidity (mg/L)	32.66 ± 4.16	22.67 ± 1.15	30.61	18.32 ± 1.51	43.92
11	Optical density (532)	0.316 ± 0.03	0.019 ± 0.00	93.04	0.021 ± 0.01	93.35
12	Turbidity (NTU)	388.16 ± 48.23	8.39 ± 1.0	97.84	13.1 ± 2.20	96.63
13	Total phosphorous (mg/L)	2.05 ± 0.10	1.61 ± 0.24	21.63	0.99 ± 0.19	51.92
14	Total nitrogen (mg/L)	20.37 ± 4.64	12.10 ± 1.19	40.61	10.49 ± 3.52	48.50
15	Chlorophyll-a (mg/m^3)	635.01 ± 86.20	15.03 ± 1.5	97.63	27.21 ± 3.07	95.72

[a]Optimized conditions: dose 15 and 10 mg/L; pH 8; rapid mixing rate 150 rpm; slow mixing rate 30 rpm; rapid mixing time 2 min; slow mixing time 5 min; settling time 20 min.

[b]Negative removal indicates an increase in the concentration of the respective parameter after treatment.

in MO extract solution are due to the use of 0.5 M NaCl solution used for preparing the extract. The reduction in total hardness was only 16.85%. The chloride concentration increases from 62 ± 1.15 to 292 ± 5.29 mg/L after the treatment of pond water. Ndabigengesere and Narasiah (1998) and Sánchez-Martín et al. (2012) observed that there was no change in chloride concentration after treatment with MO extract because they have either purified the protein from the MO extract or used tap water for extracting the protein. The reduction in alkalinity and acidity was only 11.50% and

30.59%, respectively. Ndabigengesere and Narasiah (1998) observed constant alkalinity for all doses of MO extract addition, whereas Hendrawati et al. (2016) observed little effect on the degree of acidity due to MO extract. Muyibi and Evison (1995b) and Oluduro and Aderiye (2007b) both observed a slight decrease in the acidity of treated water. The optical density, turbidity, and chlorophyll-a concentration are directly proportional to the concentration of microalgae cells present in water. The removal of microalgae cells caused a reduction in optical density, turbidity, and chlorophyll-a concentration by 93.67%, 97.83%, and 97.63%, respectively. The reduction of TP and TN was only 21.46% and 32.40%, respectively. The reduction of nutrients was low because the MO extract itself also adds nutrients to the water during treatment. Orthophosphate and nitrate do not directly involve in coagulation reaction, and because of soluble nature, its significant fraction remains in treated water (Ndabigengesere & Narasiah, 1998). The MO extract dose of 15 ml/L increased the orthophosphate, TP, and TN in distilled water to 0.118, 19.185, and 1.40 mg/L, respectively (Table 1). Ndabigengesere and Narasiah (1998) observed an increase in orthophosphate and nitrate concentration from 0.4 to 1.6 mg/L and 0.4 to 1 mg/L, respectively. MO seeds used for flocculation–coagulation resulted in >90% of bacteria and coliforms reduction (Ali, El-Taweel, & Ali, 2007; Oluduro & Aderiye, 2007a; Schwarz, 2001). Hendrawati et al. (2016) observed 80% and 45% reduction in E-coli in wastewater and groundwater, respectively. Similarly, Lürling and Beekman (2009) pointed out the potential of MO extract as an effect-oriented measure for mitigating cyanobacteria nuisance. They studied anti-cyanobacterial activity of MO seeds and reported that cyanobacteria (*Microcystis aeruginosa*) populations exhibited good growth at low doses of MO seeds powder (4–8 mg/L) but a higher dosage of 160 mg/L killed the cyanobacteria within 2 weeks (Lürling & Beekman, 2009). The dose of coagulant used in drinking water treatment is so low that the antibiotic effect of MO was not so significant (Ndabigengesere & Narasiah, 1998).

The main objective of the present study was the optimization of the coagulation process for the removal of microalgae from pond water using MO extract as a coagulant. As per Table 2, it is clear that an increase in conductivity, total dissolved solids, and chloride concentration was due to the addition of MO extract as a coagulant. Similarly, low removal of nutrients is because of the addition of soluble orthophosphate and nitrate by MO extract. Figure 2 shows that with reducing the dose of MO extract from 15 to 10 ml/L, TN and TP removal increased from 26.7% to 48.1% and 36.93% to 51.08%, respectively. However, reduced dose of MO extract reduced microalgae cell, turbidity, and chlorophyll-a removal efficiency by 0.6%, 1%, and 1.7%, respectively. Thus, by reducing the dose of MO extract and compromising with slight reduction in microalgae cell removal, higher nutrient removal can be achieved. Another way to improve nutrient removal and reduce TDS may be using active coagulating protein separated from MO extract as a coagulant. Several researchers have isolated the coagulating protein from MO extract either by dialysis, lyophilization, and ion exchange (Ndabigengesere & Narasiah, 1998; Yin, 2010). However, purification of extracted coagulating protein usually increases the cost of coagulant production and makes microalgae harvesting costly. The use of isolated protein can be proved economical only for high valued algae products. The use of MO extract along with other coagulating aids may indirectly reduce the dose of MO extract required and consequently decrease the nutrient addition to water.

Conclusions

Moringa oleifera seeds are effective natural coagulant which can be successfully applied for harvesting mixed culture of microalgae to improve the water quality of pond water. The optimum flocculation efficiency was over 93% at a dose of 15 ml/L with a slow mixing speed of 30 rpm and a settling time of 20 min. Seasonal temperature variation did not affect the harvesting of microalgae significantly. Improvement in the water quality was assessed in terms of pH, total suspended solids, total hardness, turbidity, TN, TP, and chlorophyll-a. The use of MO extract shows a good reduction in chlorophyll-a and turbidity removal while pH, alkalinity, and acidity of the water sample did not alter. However, the use of MO extract increases nutrient concentration in water. This increase in nutrients can be overcome by using purified protein and/or coagulant aids for both higher microalgae and nutrient removal. The biomass produced after coagulation can be used as raw material for biofuel generation or as fertilizers to supply nutrients in the soil.

Acknowledgment

The author would like to thank University Grants Commission (UGC), Government of India, for providing a scholarship to conduct the study.

Conflict of Interest

The authors declare that there is no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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