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Natural plant extracts as an economical and ecofriendly alternative for harvesting microalgae

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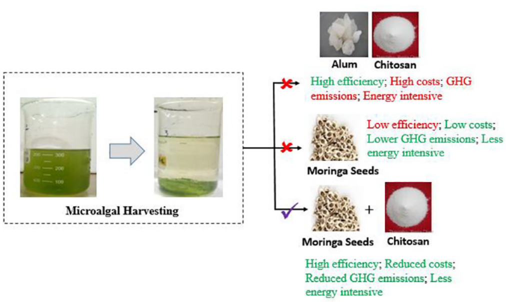
GRAPHICAL ABSTRACT



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ABSTRACT

The study investigated the ability of plant based natural coagulants from *Azadirachta indica; Ficus indica*; *Moringa oleifera*; *Citrus sinensis*; *Punica granatum* and *Musa acuminata* to harvest the microalgal biomass. Influence of eluent type (water and NaCl) and concentration (1–5 N) on coagulant extraction; coagulant dosage (1–5 g) and volume (20–100 ml); pH (6–12) and algal concentration (0.1–1 $g\,l^{-1}$) on harvesting were analyzed. The results obtained were compared with alum and chitosan. FTIR and biochemical analysis confirmed the presence of bioactive compounds to aid coagulation. Biomass removal efficiency of 75.50% was obtained with *M. oleifera* extracts (8 $mg\,ml^{-1}$) at pH 7.5–7.8, within 100 min. The harvesting efficiency increased to 95.76% when 4 $mg\,ml^{-1}$ *M. oleifera* extracts was combined with 0.75 $mg\,ml^{-1}$ chitosan. The life cycle and cost analysis acknowledged the eco-friendly coagulants as strong alternative for conventional coagulants used in microalgal harvesting, thereby improvising the overall bioprocess.

1. Introduction

Owing to the population growth and increase in standard of living in developing countries, the requirements of food/feed as well as fuel has tremendously increased over the past decade. Further, industrialization has led to the increased dependence over the fossil fuels causing the problems of global warming and environmental pollution. Microalgae are regarded as the potential feedstock for valuable range of products in a biorefinery approach ranging from renewable biofuel to value added chemicals that are expected to sort out the increasing future demands, thereby providing environmental protection and sustainable benefits (Rangabhashiyam et al, 2017; Behera et al., 2019). Phototrophic microalgae have attracted the attention of researchers, being capable of sustaining using minimal resources like wastewater and carbon dioxide from flue gases as nutrient (Behera et al., 2018a,b). However, the concentration and harvesting of microalgae occupies

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30–40% of the total process costs, thus hindering the application of the proposed technology at field scale (Wan et al., 2015). The small cell size of microalgae, lower cell concentration along with the enormous volume of culture media having densities nearly same as water, increases the cost and energy required during harvesting (Fasaei et al., 2018). The major hurdle thus lies with the process of separating the algal biomass from the water/media.

Various unit operations and approaches consisting of the chemical, mechanical and biological harvesting has been discussed by different researchers (Wan et al., 2015; Fasaei et al., 2018) which entails a huge amount of costs, energy and power consumption. Piriwitz et al., (2015) and Fasaei et al., (2018) reported that the harvesting techniques are applicable either singly or in combination depending on the physiochemical properties of dilute algal solutions. Mechanical harvesting techniques such as centrifugation, filter pressing and electrocoagulation though could be applied at ease, but requires huge amount of energy thus are not technically feasible for large scale applications as they impact the final product costs (Wan et al., 2015). To facilitate the bioprocess, the large scale algal facilities often combine the process of mechanical harvesting with that of the pre-concentration step via the use of chemical flocculants. Several research has been carried out to sediment and concentrate the microalgal biomass with the use of chemical flocculants (Wan et al., 2015; Lama et al., 2016). Twelve polyvalent salts were assessed to quantify the flocculation efficiency in *C. minutissima* culture broths by Papazi et al. (2010). Chemical coagulation process depends on the algal concentration, media pH and dosage of the flocculants (Ummalyma et al., 2016). At optimal pH and dosage of ferric chloride, > 90% biomass recovery was reported with *C. zofingiensis* (Wyatt et al., 2012). Chen et al. (2013) and Vandamme et al. (2015a,b) reported that alkali like metal hydroxides; brucite and calcite can raise the media pH resulting in significant biomass removal. Though metal salts or inorganic flocculants are efficient; the deposition of metallic residues over the biomass often adds to the downstream processing costs. Polymeric compounds or organic flocculants of biological origin have also been explored by different studies. Wan et al. (2015) reported 60% algal biomass removal efficiency with the use of polyacrylamide under optimal conditions. Chitosan also acts as an effective flocculant forming larger flocs at relatively lower dosage of (10–20) mg ml^{-1} (Blockx et al., 2018). Recent studies by Peng et al. (2017) and Huang et al. (2018) reported 99% algal recovery with the use of cationic starch at alkaline pH. Even though these polymers are touted to have relatively lesser side effects, still the chemically synthesized nature impacts the algal residues obtained during further processing into valuable products. Further, the lifecycle analysis shows higher environmental and energy impacts associated with their synthesis that adds on to the overall process costs (Zhu et al., 2018). To overcome these above said issues, natural flocculants derived from plant could act as a potential aid for harvesting algal biomass.

The natural plant extracts like Moringa seed flour, gaur gum, *Cicer arietinum* seeds, *Cactus lactifera*, fruit waste peels etc. have been applied to remove the colour and turbidity, and reduce the biological and chemical oxygen demand (BOD & COD) of highly turbid wastewaters (Kristianto, 2017). Muthuram and Sasikala (2014) reported > 90% turbidity removal from wastewater using *M. oleifera* extracts. Patchaiyappan et al. (2019) projected the requirement of optimal coagulant dosage and media pH for maximizing the turbidity removal. Though significant amount of work has been carried out with respect to the use of natural coagulants in water treatment process, the literature related to the application of this concept to recover microalgal biomass is very limited. Udom et al. (2013) investigated the effect of natural extracts of *M. oleifera* and *F. indica* on microalgal biomass recovery. Hamid et al. (2014) analyzed the effects of mixing rate and pH on flocculation efficiency of Moringa seeds to recover *Chlorella* sp. Gutierrez et al. (2015) utilized the natural extracts of ecotan™ and tanfloc™ obtained from the *Acacia mearnsii* barks for recovering 90% of microalgal biomass. Baharuddin et al. (2016) analyzed the algal biomass recovery efficiency of *M. oleifera* seeds before and after oil extraction.

In the present study, laboratory investigation was done to explore the potential of natural plant extracts as flocculants for recovering microalgal biomass. Plant extracts of neem (*Azadirachta indica*), cactus (*Ficus indica*), drumstick (*Moringa oleifera*), and waste fruit peels of orange (*Citrus sinensis*), pomegranate (*Punica granatum*) and banana (*Musa acuminata*) were utilized for harvesting the mixed microalgal consortium. Operational parameters like the eluent concentration, coagulant dosage, pH and microalgal concentration were optimized to achieve the maximal removal efficiency using jar tests. Cost analysis and life cycle assessment (LCA) was done for comparative analysis of the efficacy of the natural coagulants with that of the synthetic coagulants (alum and chitosan). Synergistic/antagonistic effect of utilizing different ratios of natural and synthetic coagulants was explored to improve the activity of natural coagulants. The proposed methodology could be utilized to recover algal biomass in an economic manner without lessening the biomass quality.

2. Methodology

2.1. Strain and growth conditions

Native microalgal consortium consisting of *Chlorella* sp., *Scenedesmus* sp., *Cynecocystis* sp., and Sp*irulina* sp. obtained from the wastewater ponds of National Institute of Technology (NIT) Rourkela were grown in 5 L Erlenmeyer flasks using 6.5% diluted source separated human urine at ambient temperature (30 ± 5 °C) with the light intensity of 205 μmol photons m^{-2} d^{-1}, in 8:16 light–dark cycle. The cultures after the exponential phase were used for flocculation studies with different coagulants.

2.2. Preparation of natural coagulants

The neem leaves, cactus pod, moringa seeds, orange, pomegranate and banana peels collected from the local area, were utilized as natural coagulants for the study. The cactus pod was washed, sliced and the gel like substance obtained was dried. The other natural coagulants were shade dried and grinded into fine powder using a household mixer. The resultant powders were sieved through 0.22 mm sieves to obtain the uniform particle size. Alum and chitosan (Himedia, India) were used as the conventional chemical coagulants for comparative analysis.

In order to obtain the active components for coagulation process, the resultant powders were dissolved with suitable concentration of sodium chloride (NaCl) [eluent] as mentioned in the next section and stirred at 100 rpm for 15 min at 37 °C, then the mixture was kept stationary for a period of 15 min. The supernatant was separated by filtration process and used further for flocculation studies. The natural coagulants extracted with NaCl (1g coagulant in 100 ml of 1 N solution) were characterized for the presence of functional groups through Fourier transform infrared spectroscopy (FTIR). The total carbohydrates in the natural plant extracts were examined through colorimetric assay using DNS method (Milley, 1959) and the monosaccharides were identified using High Performance Liquid Chromatography (HPLC) with 5 mM sulphuric acid as mobile phase, with refractive index detector. The total proteins and lipids were estimated using Bradford method (Bradford, 1976) and modified Bligh and Dyer method (1959) respectively. Phenolics content were analyzed using Folin-Ciocalteau reagent (Singleton and Rossi, 1965) with gallic acid as standard.

2.3. Experimental design for flocculation studies

To conduct the flocculation studies, a known volume of the eluent from each of the natural coagulant was added into 500 ml of microalgal broth. All the experiments were conducted in a jar test apparatus with six stirrers and a base illuminator. The cell suspension and the

Table 1
Biochemical composition of natural coagulants considered in the study.

Natural coagulants	Total carbohydrates (%)	Total proteins (%)	Total lipids (%)	Total phenolics (%)
Orange	23.65 ± 0.002	2.47 ± 0.042	0.62 ± 0.345	1.49 ± 0.070
Pomegranate	44.41 ± 0.005	1.86 ± 0.020	1.18 ± 0.595	2.23 ± 0.072
Cactus	31.77 ± 0.021	6.41 ± 0.023	1.15 ± 0.120	0.48 ± 0.035
Moringa	20.19 ± 0.003	22.07 ± 0.003	14.84 ± 0.145	0.47 ± 0.076
Neem	38.97 ± 0.029	3.22 ± 0.011	1.02 ± 0.030	1.02 ± 0.042
Banana	47.54 ± 0.010	2.21 ± 0.035	0.87 ± 0.135	0.97 ± 0.079

coagulant mixture were agitated at 150 rpm for 2 min, followed by slow mixing at 30 rpm for 20 min. After mixing, the algal flocs formed were allowed to settle for 4 h. Optical density (OD) was measured after taking 2 ml of suspension from the center of beaker without disturbing the flocs formed. Biomass recovery efficiency was calculated using the Eq. (1).

$$\%BiomassRecoveryEfficiecy = \left\{\frac{OD_{Final} - OD_{Initial}}{OD_{Initial}}\right\} * 100 \quad (1)$$

where, OD_{Final} refers to the optical density of microalgal suspension at time (t) [after settling] and $OD_{Initial}$ of microalgal suspension at time (0).

Experiments were carried out to study the effect of eluent type [NaCl/water]; eluent concentration [1–5 N]; coagulant dosage [1–5 g]; coagulant volume [20–100 ml]; pH [6–12]; microalgal concentration [0.1–1 g l^{-1}] on the algal biomass recovery. Synthetic coagulants like alum and chitosan with varying dosage of 50–250 mg ml^{-1}, pH (7.5–7.8) and algal concentration of 500 mg l^{-1} were utilized for comparative analysis.

2.4. Economic assessment and life cycle analysis

The lifecycle analysis and cost assessment was done using the data obtained from the flocculation experiments with jar test apparatus for comparative analysis of the performance of the natural and chemical coagulants. Cost analysis was done utilizing the price of chemicals (alum and chitosan) as obtained from the local vendors. 1 MT of wet algal flocs obtained was taken as the functional unit for the cost assessment and life cycle analysis. The process chain for calculation of the energy and environmental impacts of alum and chitosan has been taken from Muñoz et al. (2017). The upstream energy consumption to produce the requisite amount of alum and chitosan for removing 1 MT of algal biomass was assessed utilizing the cumulative energy demand analysis in OpenLCA software. The greenhouse gas (GHG) emissions of the process was estimated using the Tools for the Reduction and Assessment of Chemical and other Environmental Impacts (TRACI) for alum, chitosan and the natural coagulant.

2.5. Evaluation of biomass removal efficiency of flocculants mixtures

The natural flocculant showing the maximal efficiency was combined at different ratios of combination with synthetic coagulants alum and chitosan. The natural flocculant was combined with the synthetic coagulant at 20:80; 50:50 and 70:30 proportions of their optimal dosage. Jar tests were conducted to evaluate the flocculation efficiency with the culture pH of 7.5–7.8 at 500 mg l^{-1} of microalgal concentration, with settling time of 20 min. The algal flocs formed after coagulant treatments were examined through microscopic size and zeta potential analysis.

3. Results and discussion

3.1. Characterization of natural coagulants

The natural coagulants showed the presence of functional groups that possibly assist in the flocculation process. FTIR analysis was done from 400 to 4000 cm^{-1}, to analyze the presence of different active functional sites. The hydroxyl group and the polymeric stretching due to water molecule was observed at 3400 cm^{-1}, for all the plant extracts. The characteristic peaks at 3000 cm^{-1} represents the aromatic groups of C═C–H in cactus extract signifying the presence of alkanes. Sharp peaks between 1600 and 1400 cm^{-1} confirmed the presence of carboxylate groups in the chosen plant extracts. The presence of C═O stretch; C═C ring; C-O-C stretch between the bandwidth of 1400–1000 cm^{-1}, highlighted that the natural extracts from Moringa and Cactus contained aromatic compounds, polysaccharides that could help in forming dense aggregates. CN stretch between 1350 and 1490 cm^{-1} in Moringa extracts could be due to the presence of cationic moiety in the polymeric backbone. Also, the presence of carboxylate and hydroxyl groups in the FTIR spectra of all plant extracts revealed that these groups could assist in bridging thereby flocculation. Similar kind of spectrum was also reported by Vishali and Karthikeyan (2015) and Nharingo et al. (2015) for natural plant extracts used for removal of turbidity from wastewater effluents. The results obtained are also in line with the findings of Jhadav and Mahajan (2014) who had reported the presence of active functional groups to aid in coagulation process.

The presence of carbohydrates, proteins, lipids and phenolic compounds as indicated by the functional groups were also confirmed by the biochemical analysis of the plant extracts as shown in Table 1. Appreciable amount of biochemical constituents especially carbohydrates were found in all the plant extracts. Peaks from HPLC analysis showed the presence of glucose, xylose, mannose and maltose in these plant extracts. Glucose peaks were detected in *M. oleifera*, whereas strong peaks corresponding to glucose and xylose were present in cactus. Kumar et al. (2017) reported that the presence of these bioactive polysaccharides in the natural plant coagulants causes inter-particle bridging increasing the competency of flocculation process. Significant quantities of proteins and lipids were also detected in *M. oleifera* extracts compared to other natural plant extracts. Okuda et al. (2001) and Teixeira et al. (2012) also reported the presence of bioactive compounds and proteins in the saline extracts of *M. oleifera* that could potentially act as cationic polyelectrolyte thereby causing charge neutralization and resulting in algal sedimentation. The fruit peels also showed the presence of phenols that has been reported to stabilize the negatively charged algal cells via resonance (Kumar et al., (2017**)**) thus aiding in coagulation. Thus, the interaction between the natural bioactive compounds and the microalgal cells leading to stimulation of biochemical processes of charge neutralization, cross-bridging and double layer compression results in sedimentation of algal biomass.

3.2. Optimization of process parameters affecting coagulation

3.2.1. Effect of eluent concentration

The active compounds for coagulation from the natural plant components can be extracted using water and salt solution. Experiments were performed with 1 g of natural coagulants dissolved using 100 ml 1–5 N NaCl solution to check the flocculation efficiency in each of the cases. A set of experiments were also performed with only water as control under the same conditions for comparative analysis. The biomass removal efficiency of 42% and 40.5% were obtained with water as eluent (50 ml) for *M. oleifera* and *F. indica* extracts respectively. *A.*

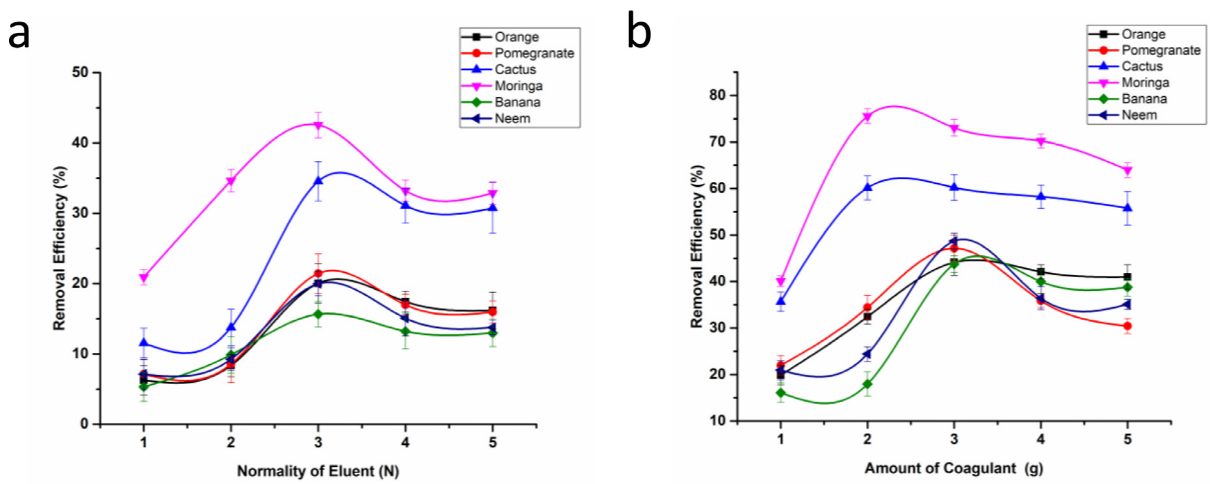

Fig. 1. a). Effect of eluent conc. on microalgal harvesting efficiency [1 g coagulant in 100 ml solution] b). Effect of coagulant amount on microalgal harvesting efficiency [3 N NaCl] at pH of 7.5–7.8.

indica; C. sinensis; *P. granatum* and *M. Acuminata* showed biomass removal efficiency of 24.5%, 12%, 14% and 11% respectively after 4 h. As illustrated in Fig. 1a, the maximal biomass removal efficiency for all the coagulants were obtained with 50 ml of 3 N NaCl solution after 4 h settling time. On increasing the concentration of NaCl, there was a gradual increase in flocculation until 3 N beyond which the efficiency was found to decline. It was evident that NaCl acts as a better eluent at optimal dosage compared to water. Studies by Okuda et al. (2001) and Vishali and Karthikeyan (2015) have also reported NaCl as a better eluent for extracting the compounds required for coagulation. Considering that active compounds for coagulation could be obtained at 3 N NaCl, this concentration was further utilized for experiments.

3.2.2. *Effect of coagulant dosage*

Coagulant dosage is an essential parameter to be considered during flocculation as overdosing or insufficient dosing might affect the treatment efficiency. Further optimization of the coagulants dosage (1–5 g) with 3 N NaCl solution (50 ml) showed the maximum biomass removal efficiency ranging from 40 to 60% for all natural coagulants at a dosage of 3 g, except for *M. oleifera* extracts which showed the highest removal efficiency of 75% at the dosage of 2 g after the settling time of 4 h (Fig. 1b). Lower the dosage of coagulant, there is an insufficient amount of bioactive compounds compared to the amount required for effective sedimentation. Beyond the optimal dosage, there was a decline in the efficiency for each of the coagulant. This might be attributed to the fact that with the concentration of NaCl being fixed at 3 N, it might not be sufficient for extraction of the coagulative compounds from the natural plant based components. Similar results were reported by Patel and Vashi (2012) and Vishali and Karthikeyan (2015) for removal of Congo dye and paint effluent respectively with the use of natural coagulants. Beyond the optimal dosage, over-deposition of the coagulants occur resulting in dispersion and restabilization, thereby declining the biomass removal efficiency (Blockx et al., 2018).

3.2.3. *Effect of pH on coagulant dose and settling time of microalgae*

pH of the culture medium plays an essential role in influencing the surface charge of the coagulants and the stability of the microalgal cells in the suspension, thus the flocculation efficiency. The physiological pH of the algal culture medium was between 7.5 and 7.8. Flocculation experiments were run at acidic (pH: 6) and higher alkaline pH (pH: 9.5 and 12), to check the efficiency of flocculation at different conditions. The coagulant volume was varied from 20 to 100 ml to check the efficiency of flocculation with different coagulant volume, with 4 h settling time. As illustrated in Fig. 2a–d, at lower pH of 6, maximum biomass removal efficiency of 63% was obtained with 60 ml of solution obtained from 2 g of *M. oleifera* extract. While at physiological pH the maximum biomass removal efficiency of 76% was obtained with 40 ml solution of 2 g of *M. oleifera* extract. The flocculation efficiency was found to be highest at pH 9.5 for all the natural coagulants. The maximal biomass removal efficiency of 85% was obtained with *M. oleifera* extract [20 ml of 2 g (3 N) solution], followed by the addition of 20 ml of 3 g extracts of *F. indica* in 3 N NaCl solution [69% efficiency]. With further increase in pH to 12, no significant increase in biomass removal efficiency was seen for the natural coagulants (Fig. 2d). Similar results were also obtained for other natural coagulants. Also it was seen that with the increase in pH, the highest amount of coagulant volume required to achieve the maximal efficiency was found to decline. With *M. oleifera*, 85% biomass removal efficiency was achieved after 60 min with pH 9.5; while maximal biomass removal efficiency of 76% was obtained after 100 min at physiological pH; and at pH of 6 biomass removal efficiency of 63% was achieved after 220 min. Thus, with the increase in pH till the optimum, the settling time for achieving the maximal removal efficiency also declined (Fig. 3).

The synergistic effects of self-flocculation as well as the charge neutralization property, occurring at higher pH plays a major role in coagulation (Blockx et al., 2018). With the gradual increase in pH of the culture, the small sized algal cells slowly aggregated into densely packed mass of cells due to increase in self flocculation property and then settled under the influence of gravity. The flocculation efficiency is based on the pH of the medium, which is also dependent over the microalgal species. The studies by Liu et al. (2013) and Maji et al. (2018) reported that a decrease in media pH could increase the flocculation efficiency in *Chlorococcum* sp. and *Scenedesmus* sp. while algal cells of *Chlorella* sp. flocculates better at alkaline pH. For most freshwater microalgae, at higher pH there is a tendency towards sedimentation and at fundamentally alkaline pH, the biomass removal efficiency increases (Teixeira et al., 2012; Blockx et al., 2018). At higher pH, as the hydroxide ion concentration increases, it combines with metallic elements mostly Mg^{2+} and Ca^{2+} present in the growth media forming precipitates (Ummalyma et al., 2016). These precipitates have positive surface charge density and large adsorptive surface area which coagulated the microalgal cells, forming denser flocs that settles under gravity, by the process known as sweep flocculation (Wu et al., 2012). Further, these precipitates also possess open type structure, with higher probability of capturing even a small microalgal biomass (Ummalyma et al., 2016). Maji et al. (2018) also recently reported that the increase in pH destabilizes the cells resulting in precipitation due to fluctuations in isoelectric point of the culture media. Thus, the combined action of increased pH, along with biochemical interaction of the cationic peptides and polysaccharides altogether improves the biomass removal

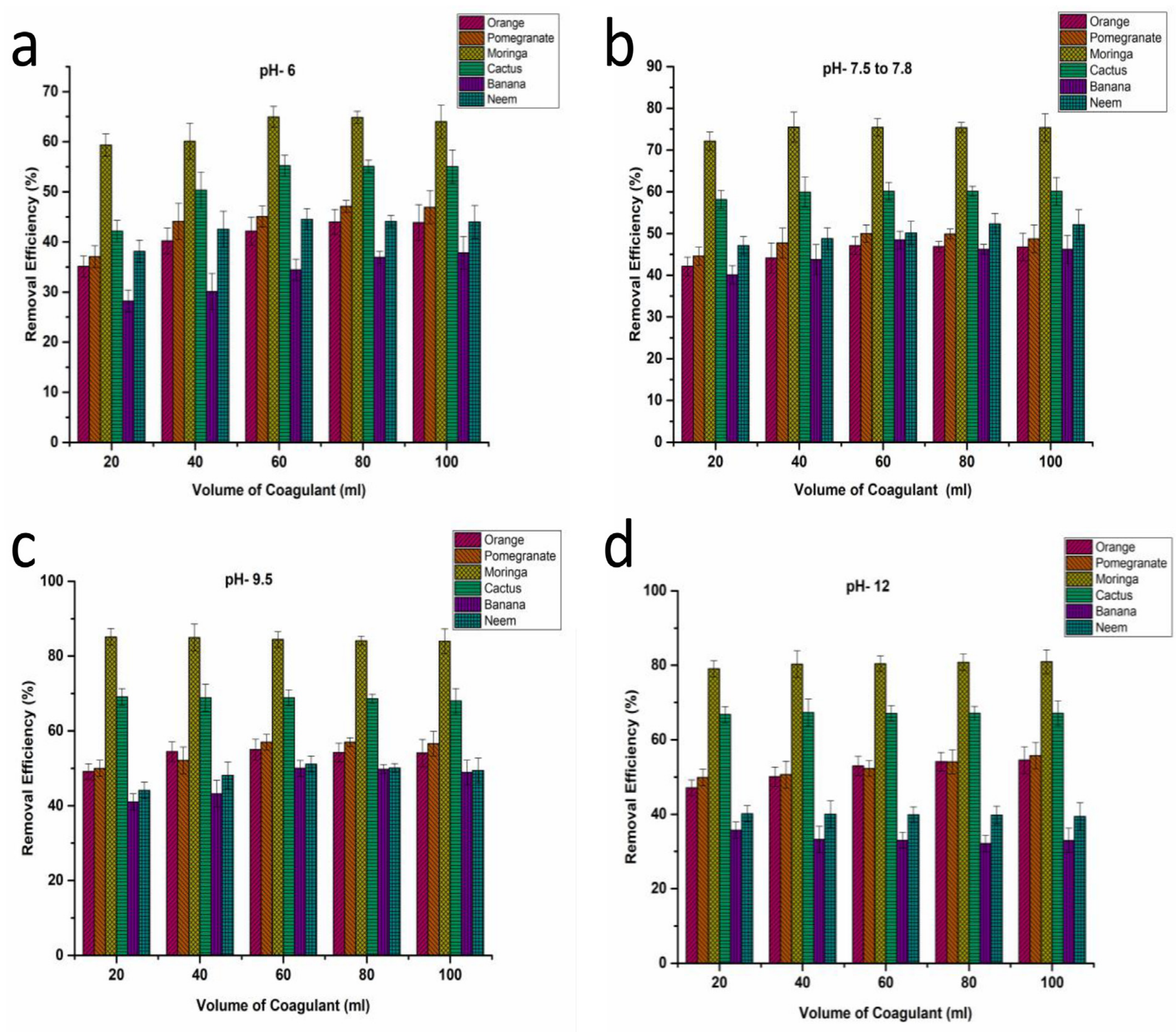

Fig. 2. Variation in microalgal harvesting efficiency over the coagulant volume and the pH of the medium. a). pH of 6b). pH of 7.5–7.8c). pH of 9.5 d). pH of 12.

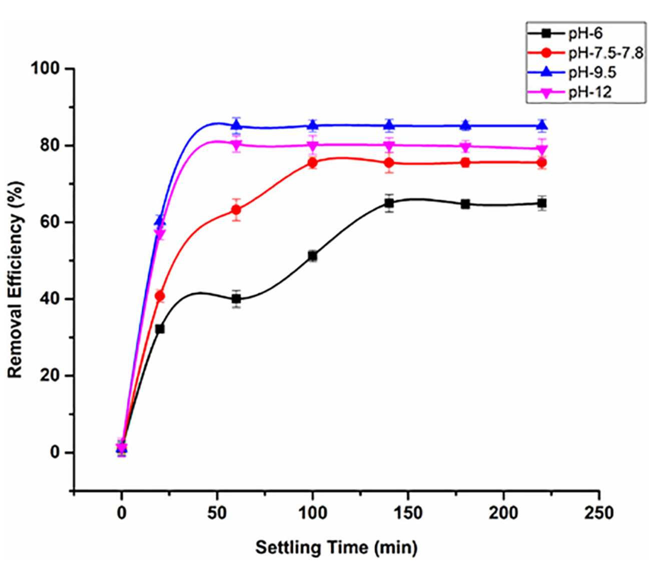

Fig. 3. Effect of pH on microalgal harvesting efficiency along with settling time for flocculation by *M. oleifera.*

efficiency. Teixeira et al. (2012) also reported significant decline in optical density of microalgal cultures with *M. oliefera* extracts at alkaline pH. Kumar et al. (2017) also reported that the combined action of sweep flocculation and the bioactive polymeric compounds could increase the efficiency of harvesting by several folds. Beyond the optimal pH, once the equilibrium is being achieved further increase in pH did not have any significant change in the biomass removal efficiency (Ummalyma et al., 2016). Sciban et al. (2009) also reported that the optimal pH of 10 for coagulation with the natural coagulants. The results are also in accordance with that of the study by Sanghi et al. (2002) with *C. anguistifolia* and Okuda et al. (2001) with *M. oleifera* who reported the maximal efficiency of these natural coagulants at alkaline pH.

3.2.4. Effect of initial microalgal concentration on coagulant dose

Microalgal concentration plays a vital role in determining the flocculation efficiency. Experiments were performed with the natural coagulant (*M. oleifera* and *F. indica*) [best two among all the natural coagulants] by varying the initial microalgal concentration (Fig. 4). A linear correlation was observed between the initial algal concentration and optimal coagulant dose for achieving the maximal biomass removal efficiency of > 75% with *M. oleifera* and > 60% with *F. indica*. With increasing microalgal concentration the amount of coagulant required to achieve the maximal flocculation was found to decline in both the

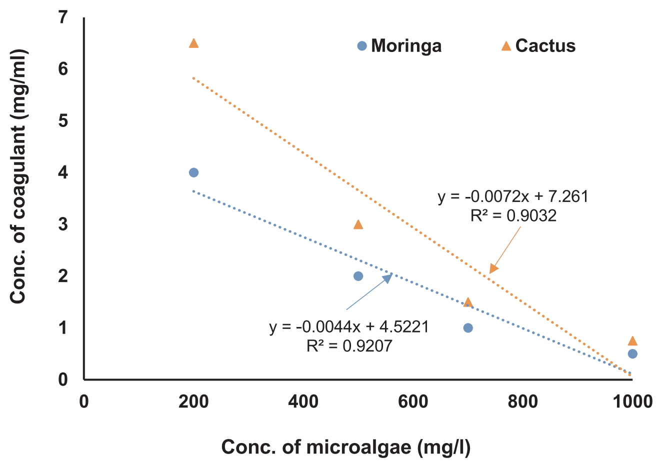

Fig. 4. Correlation between the coagulant concentration and initial algal concentration to achieve > 75% and > 60% biomass removal efficiency with *M. oleifera* and *F. indica* respectively at pH of 7.5–7.8.

cases mainly due to the mechanism of increased bridging between the algal cells thereby forming dense aggregates which settles rapidly by sweep flow mechanism under gravity. Similar findings were also reported by several authors like Papazi et al. (2010); Wyatt et al. (2012) and Udom et al. (2013), thus signifying the dependency of the flocculation process over the algal cell density in the culture media.

3.3. Comparison of the biomass removal efficiency of different coagulants

The overall summary of the coagulation studies with the jar test apparatus conducted with different natural coagulants are shown in Table 2. Synthetic coagulants like alum (inorganic) and chitosan (organic) were used as reference to compare the efficiency of the natural coagulants. All the tests were performed at the pH of 7.5–7.8 and microalgal concentration of 500 mg l^{-1}, keeping the settling time of 4 h, to evaluate the sedimentation potential of these extracts. The maximal biomass removal efficiency achieved after specific time-period has been reported in each case. In case of natural coagulants, the maximal biomass removal efficiency of 75.5% was obtained with *M. oleifera* extracts at a dosage of 8 mg ml^{-1}, with after 100 min. Maximal biomass removal efficiency of 60.12% was achieved with 12 mg ml^{-1} of *F. indica* extract after 120 min. Kumar et al. (2017) reported that under saline conditions the dimeric protein in Moringa acts as a cationic polyelectrolyte that neutralizes the negatively charged microalgal cells, thus enhancing the flocculation process. Udom et al. (2013) also reported the presence of polysaccharides and mucilage like substances in the extracts from Moringa and Cactus that assist in aggregating algal cells into dense concentrated biomass. Teixeira et al. (2012) reported the harvesting efficiency of 89% with *C. vulgaris* at pH of 9.2 with a dosage of 1 mg ml^{-1} *M. oliefera* extracts after 240 min. Biomass removal efficiency of 85% was reported for *Chlorella* sp. by Udom et al. (2013) with *M. oleifera* at the dosage of 5 mg ml^{-1}. The biomass removal efficiency with natural coagulants were reportedly lower than that of the synthetic coagulants. Maximal biomass removal efficiency of 97.78% and 96.32% were obtained with 0.05 mg ml^{-1} of alum and 0.15 mg ml^{-1} of chitosan after 10 min respectively. Variations in performance of different natural and synthetic coagulants is due to the differences in charge densities of the active functional compounds which thereby governs the process of algal bridging and stabilization of suspension, thus the overall flocculation process (Gorin et al., 2015; Zhu et al., 2018).

Table 2
Harvesting efficiency of microalgae for various coagulants under their pre-optimised conditions

Coagulant type	Optimal concentration (mg ml^{-1})	Maximal harvesting efficiency (%)	Time at which the maximal harvesting efficiency were obtained (min)
Natural coagulants			
Orange	18	47.10 ± 2.10	160
Pomegranate	18	49.97 ± 2.11	160
Cactus	12	60.12 ± 3.60	120
Moringa	8	75.50 ± 2.37	100
Neem	24	52.32 ± 2.50	140
Banana	18	48.45 ± 1.98	160
Synthetic coagulants			
Alum	0.050	97.78 ± 2.95	10
Chitosan	0.150	96.32 ± 1.03	10

*All experiments were performed with the predetermined optimal dosage of coagulants at pH of 7.5–7.8 with algal concentration of 500 mg l^{-1} for the settling time of 240 min. Time given in table represents the period at which maximal biomass harvesting were achieved and after which the efficiency becomes constant up to 240 min

3.4. Life cycle and cost analysis of different coagulants

The comparison among different coagulants were done based on the costs, environmental impacts and energy consumption. Fig. 5 illustrates the GHG emissions and the energy consumption associated with the use of natural coagulants and that of the alum and chitosan. The energy consumption and GHG emissions were found to be maximum for chitosan followed by alum. The relatively lower energy and environmental impacts of inorganic coagulant compared to the organic coagulant might be attributed to the reason that the lower requisite dosage of the inorganic coagulant to achieve the maximum biomass recovery. Economic analysis revealed that under optimal conditions, 9.02$ would be utilized to recover 1MT of wet algae with chitosan, whereas 0.28$ would be utilized to obtain 1MT wet algal biomass with alum. The cost to recover 1MT wet biomass with natural coagulant amounts to 0.037$. The costs associated for natural coagulant is due to the electricity consumption during stirring. High energy, emissions and costs associated with the organic coagulant is mainly because of the intensive production process. High cost and environmental impacts were also reported with the use of chemical coagulants by Udom et al. (2013). It is quite evident that there is a trade-off between the performance efficiency and the environmental impacts as well as energy consumption of

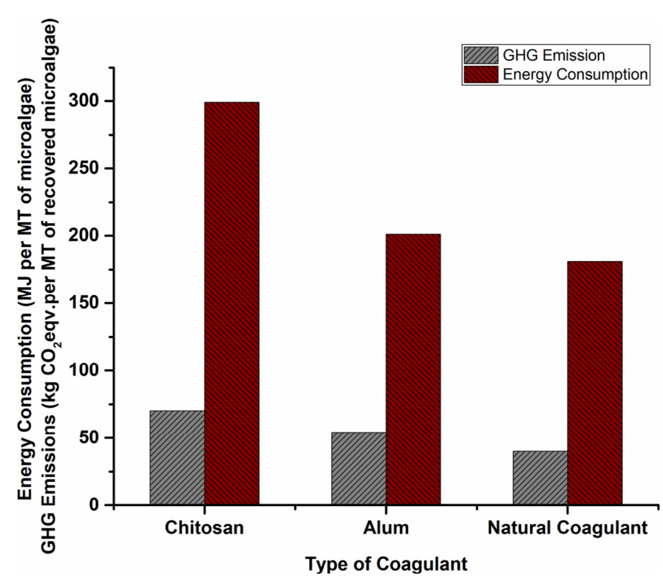

Fig. 5. Energy consumption and GHG emissions of chitosan, alum and natural coagulant (*M. oleifera*).

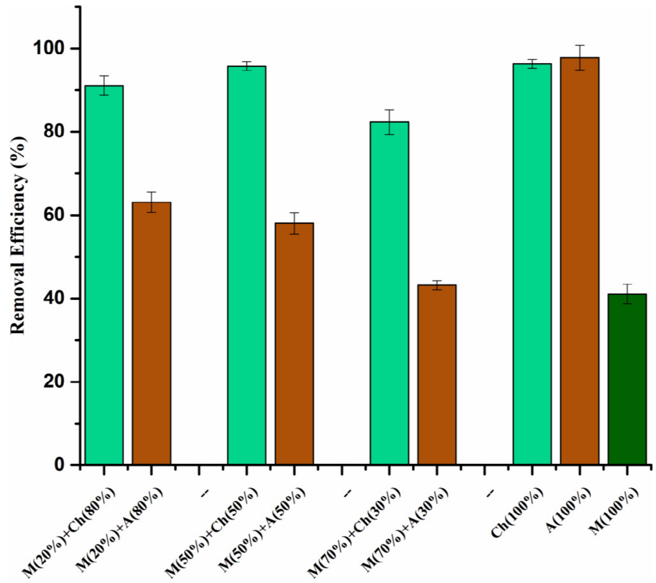

Fig. 6. Variation in biomass removal efficiency with different ratios (optimal dosage) of combined coagulants after 20 min settling time at pH of 7.5–7.8 [M: Moringa; Ch: Chitosan; A: Alum]

the natural and synthetic coagulants. Therefore, it is essential to consider both the environmental and the economic aspects of the coagulant before selecting the appropriate flocculation strategy.

3.5. Studies with the combination of natural and synthetic coagulants

Highest removal efficiency was obtained with alum and chitosan with the dosage of 0.05 mg ml^{-1} of alum and 0.15 mg ml^{-1} respectively within 10 min, however this combination when used on large scale will add to the costs and environmental impacts. Further, the use of natural coagulants even though projected lower environmental risks and associated costs, they could not match the efficiency of conventional coagulants. Thus, experiments were performed to explore the possibility of using the combination of natural and synthetic coagulants, in order to curb the disadvantages in each of the cases. As depicted in Fig. 6, interesting results were obtained while using the mixture of *M. oleifera* (best efficiency among all the natural coagulants) with alum and chitosan. Maximum removal efficiency of 95.76% was obtained with 4 mg ml^{-1} of *M. oleifera* and 0.75 mg ml^{-1} of chitosan combination after a period of 20 min. It was well comparable to that of the biomass removal efficiency of alum and chitosan used as an individual coagulating agent. As evident from the Fig. 6, the flocculation efficiency of mixture of coagulant in all ratios of combination were higher and faster compared to the pure natural extracts of *M. oleifera*. It could be well concluded that the combined effect of the natural coagulant with the chemical coagulant can effectively increase the biomass removal efficiency of the mixed algal consortium. The increase in biomass removal efficiency is due to the simultaneous mechanism of two different agents that formed gelatinous algal flocs which settles by sweep flocculation (Gorin et al., 2015). Better synergistic results obtained in case of *M. oleifera* extracts with chitosan, as both have cationic polymeric agents which neutralise the charge density of algal cells, improving the biomass removal efficiency. Further as observed through microscopic analysis, *M. oleifera* extracts destabilized the cells and chitosan assisted in formation of larger aggregates (58 μm) compared to the pure *M. oleifera* extract that settled under gravity. This was also confirmed by larger zeta potential of microalgal suspension with pure *M. oleifera* extracts showing more dispersed state compared to the combination with chitosan. Thus, the mixture of coagulants could be effectively utilized to harvest the algal biomass, ensuring greater efficiency and better process economics.

4. Conclusion

The investigation with the natural coagulants for harvesting microalgae establishes their potential to complement the conventional flocculants under optimal conditions. Among the selected natural coagulants, *M. oleifera* at the dosage of 8 mg ml^{-1} showed biomass removal efficiency of 75.5% at pH of 7.5–7.8 after 100 min. *M. oleifera* extracts (4 mg ml^{-1}) with 0.75 mg ml^{-1} of chitosan resulted in 95.76% biomass removal efficiency at the pH of 7.5–7.8 after 20 min. Thus, the partial combination of natural and the chemical flocculant would increase the flocculation efficiency, reduce the environmental and energy impacts, further improve the process economics.

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