

Evaluating the harvesting efficiency of inorganic coagulants on native microalgal consortium enriched with human urine

B. Behera, K. Nageshwari, M. Darshini and P. Balasubramanian 

ABSTRACT

Flocculation is a common technique to harvest microalgae, where the negatively charged algal cells coalesce together to form larger flocs that settle under gravity. Although several inorganic flocculants have been applied for algal biomass recovery, the dosage varies depending on the algal strain-specific features. Thus, the selection of inorganic coagulant that can be applied at a low dosage for achieving the maximal biomass recovery under normal physiological conditions is necessary. The present study analyses the influence of different inorganic flocculants like ferric chloride (FeCl_3), alum, calcium hydroxide, ferrous sulphate and copper sulphate on the biomass removal efficiency of a mixed microalgal consortium isolated from the open ponds of the National Institute of Technology Rourkela and further enriched with diluted human urine. Flocculation experiments were carried out with varying coagulant dosages, pH between 7.5 and 7.8, and 0.5 g L^{-1} algal concentration. The results revealed that FeCl_3 at the dosage of 0.05 g L^{-1} and $\text{KAl}(\text{SO}_4)_2$ with the dosage of 0.04 g L^{-1} could be utilized to achieve the biomass recovery efficiency of 99.5% and 97.9%, respectively, within a duration of 5 min. An economic evaluation of the harvesting process showed $\text{KAl}(\text{SO}_4)_2$ to be the cheapest coagulant that could be feasibly used to recover algae at a large scale.

Key words | biomass recovery, flocculation economics, harvesting efficiency, inorganic coagulants, microalgal technology

B. Behera

K. Nageshwari

P. Balasubramanian  (corresponding author)
Agricultural & Environmental Biotechnology Group,
Department of Biotechnology & Medical
Engineering,
National Institute of Technology Rourkela,
Rourkela 769008,
India
E-mail: biobala@nitrkl.ac.in

K. Nageshwari

M. Darshini

Department of Biotechnology,
Anna University,
Chennai 600025,
India

RESEARCH HIGHLIGHTS

- Harvesting efficiency of inorganic coagulants on native mixed algae was studied
- Iron and aluminium metallic salts showed the maximal flocculation efficiency
- FeCl_3 (0.05 g L^{-1}) showed a maximum of 99.56% biomass recovery in 5 min
- Sulphate salts of copper at higher dosage lessened flocculation efficiency
- Alum was found to be economically feasible with harvesting costs of $0.014 \text{ US\$ per m}^3$

INTRODUCTION

Algal technology under the biorefinery concept can be processed into fuel, as well as a range of value-added

products like pigments, biofertilizers and bioplastics (Behera & Balasubramanian 2019). However, the economic hindrances associated with the technologies related to the downstream processing and specifically the harvesting, which is responsible for the majority of costs comprising about 20–30% of the production cost. Microalgal cells are usually grown in cell suspensions/media with dilute concentrations (less than 1 g L^{-1}) and they have a density close to water (Behera & Balasubramanian 2019). They are usually fragile and small, with a size less than $30 \mu\text{m}$, and remain stable in suspension due to their negative surface charge density (Vandamme *et al.* 2015). The secretion of intracellular algogenic organic matter by the algal cell also stabilizes the particles in the culture media (Rial *et al.* 2015). The above-mentioned surface properties of algal cells in combination with their colloidal nature and low sedimentation velocity

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increases the electrostatic repulsion between the algal particles, thus stabilizing the suspension and making it difficult to separate the biomass (Chen *et al.* 2013).

Several methods are being used to recover microalgal biomass like coagulation–flocculation with inorganic, organic flocculants or even nanohydroxyapatite and co-pelletization using fungi (Vandamme *et al.* 2015). Coagulation–flocculation with inorganic flocculants is one of the easiest and most common methods of agglomerating cells/particles into clumps that would settle under gravity. The use of conventional flocculants like alum ($\text{KAl}(\text{SO}_4)_2$) and ferric chloride (FeCl_3) are widely used for separating the suspended solids/particles in the wastewater treatment process at industrial scale. The same proof of concept has been utilized to separate algal cells from the culture media (Udom *et al.* 2013). Coagulants are applied to alter the surface properties of algal cells to reduce the electrostatic repulsion in order to agglomerate them into bigger flocs. Chemical coagulants destabilize the microalgal suspensions by a series of mechanisms like adsorption and charge neutralization, sweep coagulation, cross-bridging and compression of adsorbed chains, thereby resulting in biomass recovery (Vandamme *et al.* 2015). The mechanism of coagulation can be determined by the type of interaction between the particles to be separated and the chemical coagulants used. These mechanisms also affect the floc characteristics like the floc size, structure and the resultant density. The interaction of the chemical coagulant with the microalgal cells changes the existing surface charge density/stoichiometry, resulting in charge neutralization (Kwon *et al.* 2014). Due to the addition of chemical coagulants, the water is often supersaturated to several orders of magnitude, much more than the solubility of the metal salts, resulting in the formation of large quantities of positively charged hydroxide precipitates (Vandamme *et al.* 2015). Algal cells get entrapped and embedded within the mass of solid precipitate and settle under gravity (Udom *et al.* 2013). Different metal coagulants and polyelectrolytes, during the process of hydrolysis, tend to polymerize, resulting in inter-bridge formation. Due to the cross-bridging mechanism, the diffused cells undergo destabilization and form more stable and larger flocs (Rial *et al.* 2015).

Metal salts, high molecular weight organic polymers and natural coagulants (Behera & Balasubramanian 2019) under appropriate conditions can induce coagulation. Based on the characteristics of the sample like the particle size, algal concentration, pH and ionic strength of the media, coagulants can be selected (Branyikova *et al.* 2018). Flocculation induced by positively charged metal ions like

Fe^{2+} , Al^{3+} , Ca^{2+} , Mg^{2+} have been studied extensively by Wyatt *et al.* (2012) and Vandamme *et al.* (2015). Chatsungnoen & Chisti (2016a) reported algal biomass recovery of more than 95% using *Chlorella vulgaris* concentration of 1 g L^{-1} with 250 mg L^{-1} and 275 mg L^{-1} of FeCl_3 and aluminium sulphate ($\text{Al}_2(\text{SO}_4)_3$), respectively. The study also demonstrated a variation in requirement of chemical coagulant with the algal species because *Neochloris spp.*, at 1 g L^{-1} biomass concentration, required a lower dose of 25 mg L^{-1} of $\text{Al}_2(\text{SO}_4)_3$ and 55 mg L^{-1} of FeCl_3 compared to *C. vulgaris* for achieving 95% removal efficiency. Koley *et al.* (2017) reported 80.2% and 79.2% algal biomass recovery with the use of $\text{KAl}(\text{SO}_4)_2$ for *S. obliquus* and *C. vulgaris* respectively at cell concentration of 0.54 mg L^{-1} . 91.62% flocculation efficiency was obtained with a mixed blue green algal consortia of concentration 0.381 mg L^{-1} with $\text{KAl}(\text{SO}_4)_2$ dosage of 200 mg L^{-1} (Ali *et al.* 2018). Kwon *et al.* (2011) and Chen *et al.* (2013) have compared the flocculation efficiency of the inorganic metal salts of iron, aluminium and copper. Depending on the microalgal species, characteristic size, concentration and surface charge densities, the working efficiency of different inorganic coagulants varies as depicted in the studies by Rial *et al.* (2015) and Chatsungnoen & Chisti (2016a). Even though flocculation is regarded as one of the easiest and most efficient method of harvesting, it is often associated with the generation of large amount of waste sludge and the accumulation of residual ions over the algal biomass. This also increases the cost economics associated with the by-product disposal and further downstream processing into subsequent products. For a specific algal consortium, the amount of coagulant required and the flocculation efficiency obtained might also vary. Thus, it is essential to identify the necessary coagulant that could be applied at the lowest dosage for a specific microalgae in order to achieve the maximal biomass removal efficiency within a shorter settling time.

The present study attempts to make a preliminary estimation of the flocculating potential of the different inorganic flocculants for harvesting a mixed native algal consortium isolated from the ponds of the National Institute of Technology (NIT) Rourkela. The commonly used inorganic flocculants such as FeCl_3 , $\text{KAl}(\text{SO}_4)_2$, calcium hydroxide ($\text{Ca}(\text{OH})_2$), ferrous sulphate (FeSO_4) and copper sulphate (CuSO_4) were used for flocculating the algal biomass, which was enriched in 6.5% (v/v) diluted urine. Under the physiological medium pH, the requisite dosage for achieving the maximal biomass removal was evaluated along with the flocculation time. The economic evaluation of the costs

associated with the use of these inorganic metallic salts has also been illustrated. Very limited studies have been carried out with regard to the use of chemical flocculants for harvesting microalgae cultured with diluted human urine. Since the specific characteristics of microalgae vary, such studies are essential for understanding the relative effect of different coagulant concentration on algal harvesting efficiency before proceeding to their large-scale utilization. Algae-specific coagulant dosage would not only help to reduce the residual ion concentration over the algal biomass but would further aid in reducing the associated process costs.

METHODOLOGY

Algal strain and culture conditions

A mixed microalgal consortium from the open ponds of the NIT Rourkela was collected. The microalgal culture was grown in BG11 medium with 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at ambient temperature ($30 \pm 5^\circ\text{C}$) with a photoperiod of 8:16 light–dark cycle. The mixed microalgal consortium was enriched and acclimatized in diluted human urine (6.5% v/v), with 205 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with periodic exposure of 8 h light and 16 h dark conditions at an ambient temperature of $30 \pm 5^\circ\text{C}$.

The sample of culture broth was serially diluted with the fresh medium and the optical density was measured at 680 nm. Biomass concentration was evaluated spectrophotometrically using Equation (1) by correlating the increase in absorbance with that of biomass content using the correlation curve with a slope of 0.8429 and a regression coefficient (R^2) of 0.988.

$$\text{Biomass content (mg mL}^{-1}\text{)} = \frac{\text{OD}_{680} \times \text{Dilution factor}}{\text{Slope}} \quad (1)$$

The specific growth rate and biomass productivity of microalgae were estimated using Equations (2) and (3), respectively.

$$\text{Specific growth rate (per day)} = \frac{\ln X_t - \ln X_0}{t} \quad (2)$$

$$\text{Biomass productivity (mg L}^{-1}\text{per day)} = (X_t - X_0)/t \quad (3)$$

where, X_t is the cell concentration in mg L^{-1} after time t (days) and X_0 is the initial cell concentration expressed as mg L^{-1} .

The algal species in the culture media were identified up to their genus level based on the corresponding morphology at 100 \times magnification using a compound bright field microscope.

Flocculation studies

Selection of coagulants

Chemical inorganic flocculants have been effective in removing algal biomass; however, the dosage of these coagulants varies based on the nature of microalgae. A preliminary study was therefore carried out with the reported conventional chemical coagulants under the physiological conditions without varying the operating parameters. Inorganic coagulants like FeCl_3 , $\text{KAl}(\text{SO}_4)_2$, $\text{Ca}(\text{OH})_2$, FeSO_4 and CuSO_4 were utilized in the present study. All the coagulants (chemicals) used were analytical grade and were procured from Himedia. The dosages of different coagulants were selected based on previous literature (Kwon *et al.* 2011; Chen *et al.* 2013; Rial *et al.* 2015; Chekli *et al.* 2017). Concentration of FeCl_3 (Kwon *et al.* 2011; Chekli *et al.* 2017; Loganathan *et al.* 2018); $\text{Al}_2(\text{SO}_4)_3$ (Loganathan *et al.* 2018); $\text{Ca}(\text{OH})_2$ (Kwon *et al.* 2011; Chen *et al.* 2013); CuSO_4 (Rial *et al.* 2015) and FeSO_4 (Reyes & Labra 2016) were selected from the previous studies. The range of selected coagulants have been applied for various algal strains cultivated in different media. The same inorganic coagulant within the prescribed range was utilized to test the efficiency of flocculation for the native mixed microalgal strain isolated from the open ponds of the NIT Rourkela and further enriched with diluted urine media. Most of the studies as cited above used a single microalgal species. Since the mixed algal consortium contained microalgal species similar to the above-mentioned studies, a comparative analysis of the harvesting efficiency with different inorganic flocculants was done to identify the suitable coagulant to be used for maximal algal biomass recovery within a shorter flocculation time-period.

Jar test experiments

The influence of flocculant type on biomass recovery was analyzed through routine flocculation studies using a six base jar test apparatus with light illumination at the bottom. A sample of 400 mL (80% of the working volume) of microalgal suspension was taken in 500 mL beakers. The culture media was stirred rapidly at 150 rpm for 2 min with the addition of a suitable dosage of chemical coagulant. Slow mixing was carried out by reducing the stirring speed

to 30 rpm over a period of 20 min to allow the flocs to form. The flocs formed were allowed to settle and the flocculation time was noted in each case. A 2 mL of the supernatant was sampled in a falcon tube to obtain the optical density at 680 nm every 5 min for a period of 1 h. The flocculation efficiency was obtained using Equation (4), as given by Rial et al. (2015) and Behera & Balasubramanian (2019). All experiments were carried out in triplicates and the results were represented in mean with standard deviation.

$$\text{Flocculation efficiency (\%)} = \left\{ \frac{B - A}{B} \right\} \times 100 \quad (4)$$

where B and A are the initial and final absorbance of the sample at 680 nm, respectively.

The physiological pH of the algal suspension was between 7.5 and 7.8 and the algal concentration was kept constant at $0.50 \pm 0.05 \text{ g L}^{-1}$ because the algal culture for the jar test studies used were taken from a 30-L batch open photobioreactor. The present study is confined within the limits to utilize the pre-optimized reaction conditions of inorganic coagulant dosage for flocculation experiments without varying the other parameters such as pH, microalgal species and its concentration.

Evaluation of the harvesting costs associated with the inorganic coagulants

The optimal dosage and the maximal efficiency obtained for each of the inorganic coagulants was utilized as the baseline information along with the unit costs (cost of coagulant (US \$ per g)) to estimate the expenditure required to harvest 1 m^3 of microalgal broth. The price of the coagulants has been considered based on the bulk price of the chemicals of commercial or industrial grade. The cost of harvesting microalgae was calculated using the following equation Equation (5) as outlined in Rial et al. (2015).

$$\begin{aligned} \text{Cost of harvesting (US \$ per m}^3\text{)} = & \\ & \left\{ \text{Coagulant dose (g L}^{-1}\text{)} \times \text{Flocculant price (US \$ per g)} \right. \\ & \times \left(\frac{100}{\% \text{ Flocculation efficiency}} \right) \times 1,000 \left. \right\} \quad (5) \end{aligned}$$

RESULTS AND DISCUSSION

The flocculation studies with the use of inorganic coagulants have been quite common in water treatment. However, to

harness the native mixed microalgal consortia grown on specific media composition, algal-specific flocculation studies have to be explored. As the biomass recovery efficiency depends on the species-specific features of the microalgae, the flocculation potential of different inorganic metal salts were estimated in the present study. The results obtained in each case were also compared with the previous studies reported in literature. This study presents the rationale behind the variations in the flocculation efficiency of microalgal from the open ponds of the NIT Rourkela, which were enriched with diluted human urine using different inorganic metal salts in the subsequent subsections.

Algal characteristics and growth analysis

The microscopic image showed the presence of single large coccoid cells, smaller circular cells aggregated as clumps, colonial cells and filamentous-branched structures with cylindrical cells. The microalgal consortium isolated from the open ponds of the NIT Rourkela thus consisted of mainly *Chlorella* sp., along with *Scenedesmus* sp., *Synechocystis* sp. and *Spirulina* sp. (Figure 1(a)) as observed by microscopic examination at 100× magnification. Figure 1(b) shows the growth pattern of microalgal consortium in diluted urine. The specific growth rate of microalgae in urine media was found to be 0.26 per day with 6.5% (v/v) diluted urine media. The biomass productivity was found to be 211 mg L^{-1} per day with a biomass content of $2,520 \text{ mg L}^{-1}$. Tuantet et al. (2014) reported the biomass content of $9,800 \text{ mg L}^{-1}$ of *Chlorella sorokiniana* in a short path photobioreactor with 2–3 times diluted urine supplemented with Mg^{2+} ions. Similar studies have also been done by Chang et al. (2013) and Jaatinen et al. (2016) using *Spirulina platensis* and *C. vulgaris*, which showed a biomass content of 800 mg L^{-1} and 730 mg L^{-1} , respectively using diluted urine as the nutrient media. A similar approach was followed while growing the mixed algal culture because the earlier reported studies also showed related algal species. The difference in the yield obtained in the present study and the cited literature might be due to the variations in the urine type, the nutrient content and other operational as well as environmental conditions. The biochemical and physiochemical properties of microalgae, the nutrient availability in urine and the acclimatization by microalgae for the nutrient sources is also responsible for the differences in the microalgal growth rate.

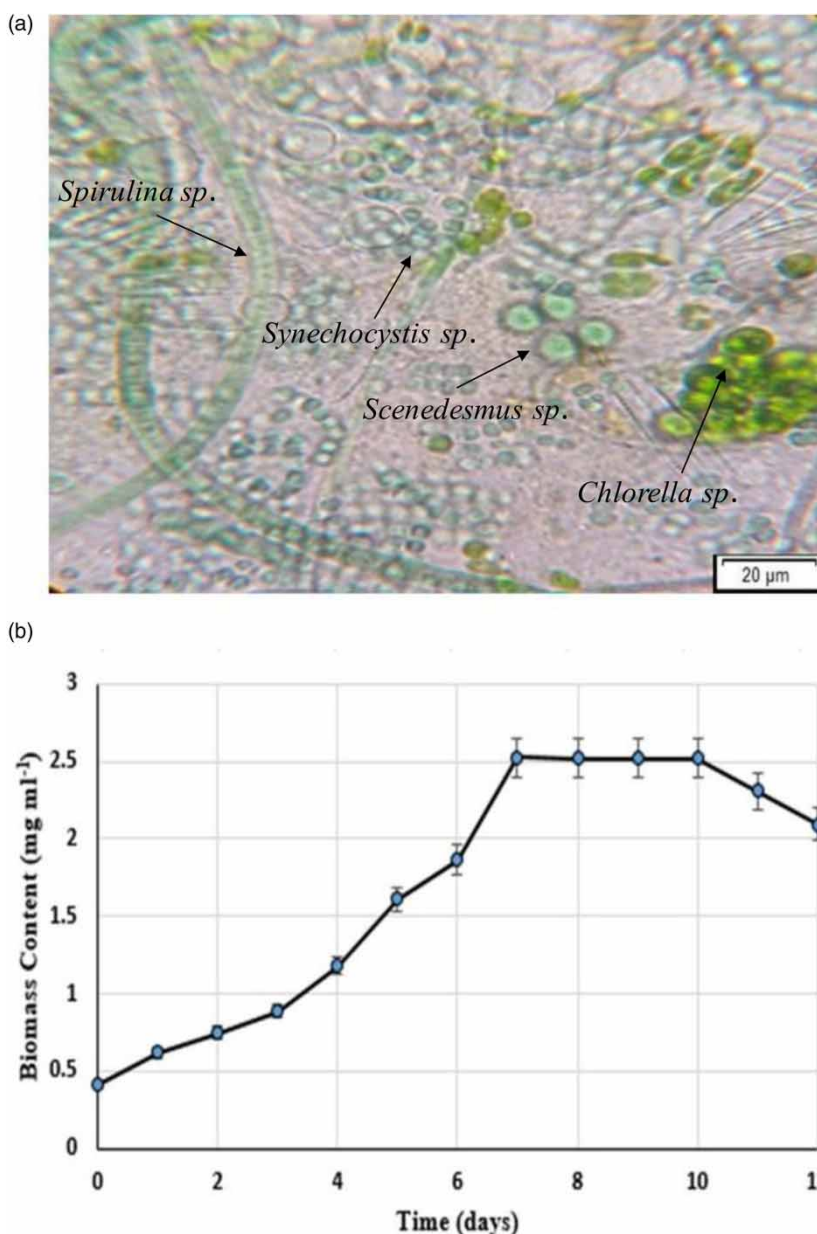


Figure 1 | (a) Microscopic image of microalgal consortium from the open ponds of the NIT Rourkela and (b) growth curve of a microalgal consortium with diluted urine (6.5%) as a nutrient source.

Flocculation efficiency of inorganic coagulants

Ferric chloride

Ferric chloride (FeCl_3) showed the maximum efficiency of 99.5% at physiological pH 7.5–7.8 with 0.05 g L^{-1} concentration (Figure 2(a)). Udom *et al.* (2013) reported the highest efficiency for FeCl_3 at 93% with a dosage of 122 mg L^{-1} at pH 6 for *Chlorella zofingiensis*.

Chatsungnoen & Chisti (2016a) also reported that more than 95% of the microalgal biomass can be removed at the dosage of 250 mg L^{-1} FeCl_3 . It was found that with the increase in FeCl_3 dosage from 12.5 mg L^{-1} to 62.5 mg L^{-1} , the biomass removal efficiency increased from 62.5% to 99.5%, beyond which it decreased to 96%. The metallic salts of iron mostly use the mechanism of charge neutralization (Branyikova *et al.* 2018). The study by Wyatt *et al.* (2012) reported that the precipitates of ferric hydroxide

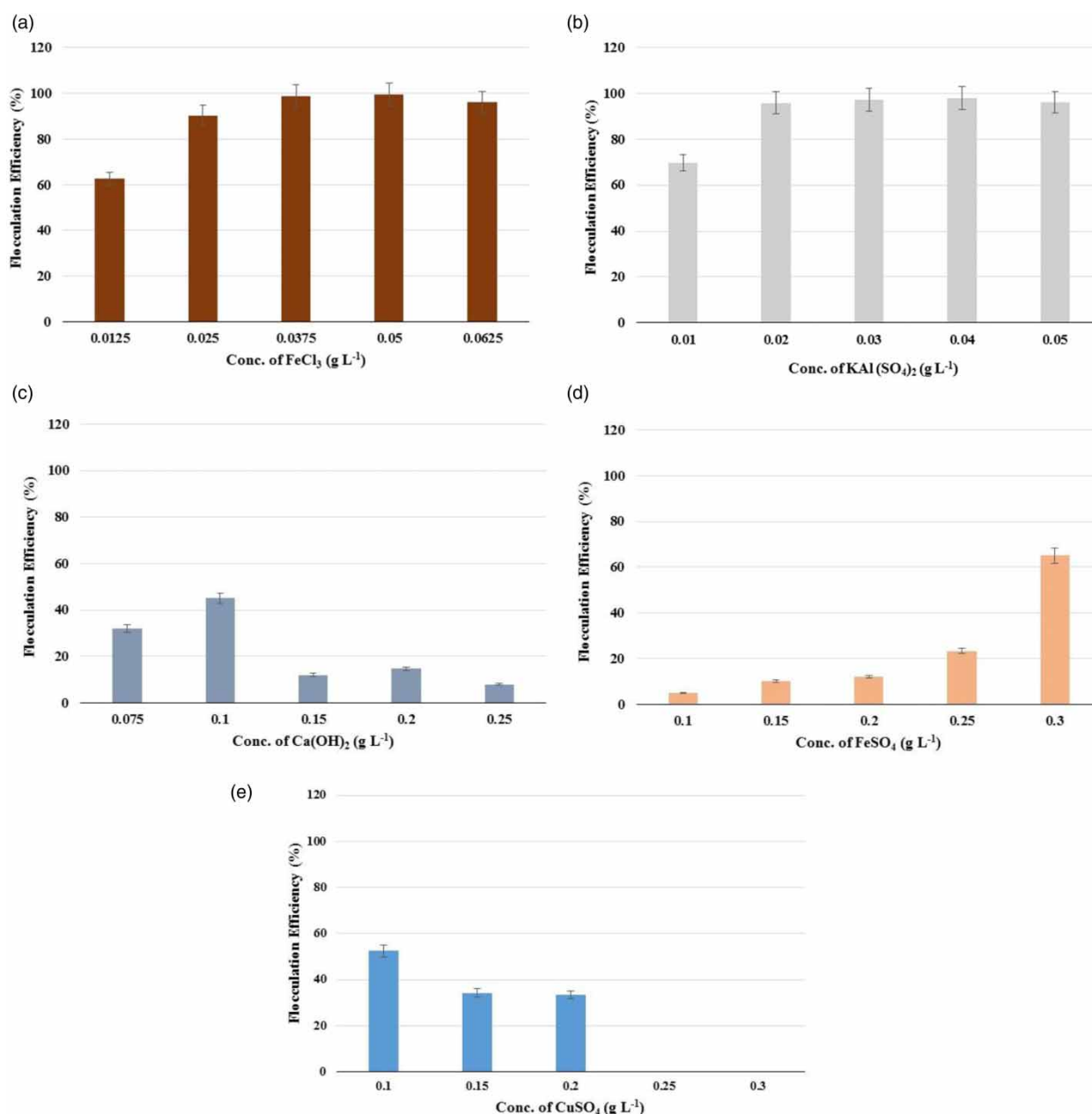


Figure 2 | Flocculation efficiency of harvesting microalgae: (a) FeCl₃, (b) KAl(SO₄)₂, (c) Ca(OH)₂, (d) FeSO₄ and (e) CuSO₄.

(Fe(OH)₃) are 10 times smaller than that of the microalgal cells. Most studies utilizing ferric ions reported higher harvesting efficiency due to the high surface charge density of these ions (Udom *et al.* 2013; Branyikova *et al.* 2018). The positively charged precipitate is attracted to the negatively charged microalgal cells, below the requisite dosage, and so the volume of precipitates formed might not be sufficient to flocculate the algal cells. Thus, an optimal

concentration of FeCl₃ is required to obtain the maximal flocculation.

Alum

Alum (KAl(SO₄)₂) is one of the most commonly used coagulants for flocculation studies. The flocculation efficiency of KAl(SO₄)₂ for harvesting the mixed algal

consortium is shown in Figure 2(b). $\text{KAl}(\text{SO}_4)_2$ showed the maximum efficiency of 97.9% at the dosage of 0.04 g L^{-1} with pH 7.5–7.8. Similar to the above study, Loganathan et al. (2018) used 30 mg L^{-1} of $\text{KAl}(\text{SO}_4)_2$, resulting in 96% microalgal removal efficiency at pH 8.2. A slight decline in biomass removal efficiency was observed at 0.05 g L^{-1} . Chen et al. (2013) reported 93% biomass removal efficiency with *Scenedesmus* sp., with an 0.3 g L^{-1} $\text{KAl}(\text{SO}_4)_2$ dosage. Biomass removal efficiency of 91% was reported for microalgae with 0.25 g L^{-1} $\text{KAl}(\text{SO}_4)_2$ (Koley et al. 2017). Ali et al. (2018) reported 91.6% maximal microalgal biomass removal efficiency with 0.2 g L^{-1} $\text{KAl}(\text{SO}_4)_2$ for *Conocarpus erectus*. The variation in pH and dosage, as well as the biomass removal efficiency, might be attributed to the microalgal species under consideration. Branyikova et al. (2018) reported that the concentration of aluminium salts required for charge neutralization depends on the size and surface characteristics of the algal cells. Similar to the ferric ions, aluminate ions also have a high surface charge density, which can be used to achieve significant microalgal recovery (Loganathan et al. 2018). Maintaining an optimal concentration of coagulant is essential to achieve the desired harvesting efficiency without unwanted contamination of the culture media.

Calcium hydroxide

Calcium hydroxide ($\text{Ca}(\text{OH})_2$) precipitates with the increase in pH that coagulates microalgal cells resulting in formation of stable agglomerates. Calcium ions with phosphate in the media often form calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) precipitates that have a positive surface charge, and are thus involved in the process of charge neutralization (Branyikova et al. 2018). For the current study, $\text{Ca}(\text{OH})_2$ concentration varied in the range of 0.075 – 0.25 g L^{-1} and the biomass removal efficiency is shown in Figure 2(c). It was found that at the dosage of 0.1 g L^{-1} , a maximal flocculation efficiency of 45% was achieved. Chen et al. (2013) reported a 90% biomass harvesting efficiency with *Scenedesmus* sp. using 0.3 g L^{-1} $\text{Ca}(\text{OH})_2$. With a further increment in concentration, the microalgal biomass removal efficiency was found to decline gradually until 8%. The study by Vandamme et al. (2015) reported that significant calcium salt precipitation results in flocculation efficiency of 90% at a pH ranging from 10 to 10.5. Since the present study used a physiological pH much lower than 10, a lower flocculation efficiency was observed compared to the other cited literature.

Ferrous sulphate

Sulphate salts of iron are also expected to cause algal flocculation due to charge neutralisation. Maximal microalgal biomass removal efficiency of 65% was obtained with 0.3 g L^{-1} of FeSO_4 , as represented in Figure 2(d). The removal efficiency was found to increase with an increase in coagulant dosage. The dosage was restricted to 0.3 g L^{-1} because iron (metal) salts at higher dosage are often associated with corrosive effects (Branyikova et al. 2018). With the increase in coagulant dosage, the amount of Fe^{2+} available will be sufficient to cause charge neutralisation for achieving a significant biomass removal efficiency. Similar to the present study, Reyes & Labra (2016) reported a maximal microalgal biomass removal efficiency of 69% with 1.5 g L^{-1} FeSO_4 . A flocculation efficiency of 40% has been reported by Kwon et al. (2011) using sulphate salts of iron for separating *Dunaliella tertiolecta*. During water treatment, FeSO_4 dosage usually varies between 5 and 50 mg L^{-1} ; however, the optimal dosage is dependent on the media conditions (Pal 2017). The study also reported that increasing the concentration of iron sulphate salts might result in unwanted corrosion at a real-time industrial scale, and thus would increase the maintenance costs. It is therefore not advisable to use a higher dosage of iron sulphates for microalgal biomass recovery.

Copper sulphate

The positively charged cations in CuSO_4 could be used for reducing the repulsion between the negatively charged algal cells resulting in formation of flocs. It is evident from Figure 2(e) that as the dosage of CuSO_4 increased, the microalgal biomass recovery was found to decline. Microalgal biomass removal efficiency of 52.4% was reported with 0.5 g L^{-1} of CuSO_4 . With a further increase in the dosage to 2 g L^{-1} , the algal harvesting efficiency declined to 33.3%. Following a gradual increase in dosage, no significant biomass removal efficiency was observed. Flocculation efficiency depends on the coagulant dosage as well as the algal cell abundance (Udom et al. 2013). The concentration 0.25 and 0.3 g L^{-1} of CuSO_4 did not show any flocculation because a higher dosage resulted in contaminating the solution and thus resulting in the loss of algal biomass.

Similar to the above study, Rial et al. (2015) reported the microalgal biomass removal efficiency of nearly 60% with 0.5 g L^{-1} CuSO_4 , with no significant change in harvesting efficiency with a further increase in coagulant dosage. In contrast to the present study, Vera Morales et al. (2016)

reported the flocculation efficiency of CuSO_4 to be less than the alkaline agent Ca(OH)_2 . Studies by Rial *et al.* (2015); Vera Morales *et al.* (2016) and Kansole & Lin (2017) reported that using a higher dosage of CuSO_4 can have toxic effects and result in residual deposition of ions over the algal biomass, thereby increasing the downstream processing costs. It was also observed in the present study that with the increase in concentration of CuSO_4 , the supernatant solution after flocculation was found to be blue in colour. The colour retained by the culture media is expected to hinder the reuse/recycle of the culture medium. Hence, the use of a high concentration of CuSO_4 during biomass harvesting is limited.

Comparative efficiency of different flocculants in terms of flocculation time-period

The efficiency of different inorganic coagulants in terms of the dosage, biomass recovery efficiency and flocculation time (minimum time to achieve the highest efficiency beyond which it remained constant for 1 h) is shown in Table 1. Among all the inorganic coagulants it was found that FeCl_3 (0.05 g L^{-1}) showed the maximal biomass removal efficiency within 5 min, beyond which it remained constant over a period of 1 h. $\text{KAl(SO}_4)_2$ at a dosage of 0.04 g L^{-1} showed an algal harvesting efficiency of 97.9% within 5 min. For Ca(OH)_2 , the dosage of 0.1 g L^{-1} resulted in maximal removal efficiency of 45% after 20 min. Inorganic salts of FeSO_4 and CuSO_4 with a dosage of 0.3 g L^{-1} and 0.5 g L^{-1} showed the maximal harvesting efficiency of 65% and 52.4% within the flocculation time of 20 min and 30 min, respectively.

Different researchers, including Chen *et al.* (2013), Vandamme *et al.* (2015) and Chatsungnoen & Chisti (2016a) have used several multivalent metal cations for flocculating algal biomass. The trend of flocculation for FeCl_3 and $\text{KAl(SO}_4)_2$ in the present study was found to be similar to the study reported by Reyes & Labra (2016). The algal

harvesting efficiency was almost constant after the dosage of 0.02 g L^{-1} and 0.0375 g L^{-1} for $\text{KAl(SO}_4)_2$ and FeCl_3 , respectively. Both Al^{3+} and Fe^{3+} in H_2O are hydrated to a certain extent and have a hydration shell with six octahedrally coordinated water molecules. The soluble aluminate and ferric metal ion species play an important role during the coagulation process (Reyes & Labra 2016). Aluminium and iron salts provide cationic hydrolysis products that adsorb onto the microalgal cells causing charge neutralization (Chen *et al.* 2013). Both the metal ions have high surface charge density compared to other metallic salts. The solubility of ferric ions is higher at a broader range of pH, compared to aluminate ions that work at pH 6. Lower concentration of Al^{3+} ions could therefore precipitate with hydroxyl ions and achieve a higher flocculation efficiency compared to ferric ions. Destabilization depends on the algal concentration and their electrophoretic mobility, and thus an optimal concentration of positively charged hydrolysing ions is necessary to cause charge neutralization by adsorption onto the surface of negatively charged algal cells. Both the inorganic coagulants produce trivalent metal ions with high surface charge density, and the cell-to-cell collisions in the suspension therefore increases and hence the combination of these coagulants is expected to improve the flocculation efficiency (Loganathan *et al.* 2018).

Equilibrium is often achieved after an optimal dose; hence, there is no further significant increase in flocculation efficiency beyond it (Branyikova *et al.* 2018). The efficiency of flocculation and the flocculation time depends on the characteristic features of the coagulant along with the biomass to be harvested. The efficiency reported for FeCl_3 is comparable to that of the removal efficiency of over 90% as reported studies by Wyatt *et al.* (2012) and Chatsungnoen & Chisti (2016a). It has also been reported that at the media pH ranging from 7 to 8, the positively charged Fe(OH)_3 precipitates and monomeric hydroxy and ferric cations dominate, which often results in significant charge neutralization. $\text{KAl(SO}_4)_2$ has also proven to be an efficient coagulant for different microalgal cultures, in different dosages ranging from 50 to 300 mg L^{-1} . With *Scenedesmus* sp., Chen *et al.* (2013) reported flocculation efficiencies of 97.3%, 94.9% and 90% with FeCl_3 after 2 min, $\text{KAl(SO}_4)_2$ over 10 min and Ca(OH)_2 after 120 min, respectively. Rial *et al.* (2015) reported the maximal biomass efficiency was achieved with 200 mg L^{-1} of metal salts after 510 min for *Chaetoceros gracilis*. Due to the difference in surface charge density of microalgal cells, there is a variation in requisite concentration of inorganic coagulant to achieve the desired flocculation efficiencies at the appropriate flocculation time.

Table 1 | Comparison of flocculation efficiency of different inorganic coagulants

Coagulant	Coagulant dosage (g L^{-1})	Flocculation time (min)	Max. removal efficiency (%)
Ferric chloride (FeCl_3)	0.05	5	99.5
Alum ($\text{KAl(SO}_4)_2$)	0.04	5	97.9
Calcium hydroxide (Ca(OH)_2)	0.10	20	45.0
Ferrous sulphate (FeSO_4)	0.30	30	65.0
Copper sulphate (CuSO_4)	0.50	20	52.4

Evaluation of economics of algal harvesting using inorganic salts

Algal harvesting occupies 20–30% of the total process costs (Vandamme *et al.* 2015); therefore, it is essential to analyse the costs of using these inorganic coagulants for recovering algal biomass (Table 2). It could be concluded that among all the inorganic coagulants, $\text{KAl}(\text{SO}_4)_2$ is the cheapest (0.014 US\$ per m^3 of algal culture broth). FeCl_3 with 99.5% flocculation efficiency (highest) incurs harvesting costs of 0.022 US\$ per m^3 . The costs associated depends on the dosage of the coagulant used as well as the flocculation efficiency (Rial *et al.* 2015). Inorganic coagulants like $\text{KAl}(\text{SO}_4)_2$ could be used efficiently for harvesting algal biomass at a large scale. The low dosage with higher efficiency is expected to be commercially affordable with relatively lesser contamination issues for processing the algal biomass into biofuel.

The residual deposition of flocculants over the algal biomass influences the yield of products, and their subsequent downstream processing and overall cost economics. Borges *et al.* (2011) reported that the anionic flocculants increase the saturated fatty acid content of microalgae. Cationic flocculants, on the other hand, increased the extractability of unsaturated fatty acids. Rwehumbiza *et al.* (2012) reported that the use of $\text{KAl}(\text{SO}_4)_2$ during flocculation did not interfere with the quality of fatty acid methyl esters and the residual aluminium levels were found to be very low. Use of alkaline coagulants like NaOH increased the concentration of saturated fatty acids and decreased the levels of polyunsaturated C20:4 and C20:5 fatty acids (Borges *et al.* 2016). Borges *et al.* (2011) reported that the residual flocculants on the algal surface form inter-bridges and loops, thereby helping trap special complex lipids by weak London forces, which influences the fatty acid profile. The utilization of cationic and alkaline flocculants also reduces the content of polyunsaturated fatty acids, thereby

producing biodiesel with better oxidative stability (Borges *et al.* 2016). Chatsungnoen & Chisti (2016b) reported that the residual flocculants over the biomass did not influence the recovery of total lipids during solvent extraction irrespective of the algal species used. As the present study was carried out to process the biomass for biofuel, the use of $\text{KAl}(\text{SO}_4)_2$ might prove to be advantageous in terms of the biomass recovery efficiency, as well as the quality and quantity of product to be derived, without incrementing the associated costs.

CONCLUSION

The dosage of flocculants and the resultant biomass recovery efficiency depends on the species-specific features of the microalgae. The present study evaluated the flocculation efficiency of FeCl_3 , $\text{KAl}(\text{SO}_4)_2$, $\text{Ca}(\text{OH})_2$, FeSO_4 and CuSO_4 for harvesting a native microalgal consortium enriched with diluted human urine. FeCl_3 and $\text{KAl}(\text{SO}_4)_2$ showed maximal flocculation efficiency. The study found that 0.05 g L^{-1} FeCl_3 over a flocculation time of 5 min resulted in a biomass harvesting efficiency of 99.5% at the physiological pH with an algal concentration of 0.5 g L^{-1} , and 0.04 g L^{-1} of $\text{KAl}(\text{SO}_4)_2$ showed harvesting efficiency of 97.9% within 5 min. Other metal salts could reach a maximum (45–65%) of biomass removal efficiency with a higher flocculation time. Estimation of the harvesting costs showed $\text{KAl}(\text{SO}_4)_2$ to be commercially feasible for harvesting the algal consortium at a large scale. Such studies are essential for identifying the requisite coagulant and the subsequent dosage with appropriate flocculation time for achieving the maximum biomass recovery without altering the physicochemical parameters of the microalgal culture at low cost and less time.

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Table 2 | Harvesting costs in dollar (\$) per m^3 of algal culture broth

Inorganic coagulant	Price of coagulant (US\$ per gram)	Process cost (US\$ per m^3)
Ferric chloride (FeCl_3)	4.48×10^{-4}	0.022
Alum ($\text{KAl}(\text{SO}_4)_2$)	3.36×10^{-4}	0.014
Calcium hydroxide ($\text{Ca}(\text{OH})_2$)	1.26×10^{-4}	0.028
Ferrous sulphate (FeSO_4)	1.40×10^{-4}	0.065
Copper sulphate (CuSO_4)	2.59×10^{-3}	2.471

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