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Establishing the nexus between the coagulant for microalgae harvesting and the biomass nutrient assemblage

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Microalgae biomass is being studied as a potential resource for the production of renewable biofertilizer, but transforming the highly dispersed miniscule microalgae cells into harvestable biomass is challenging. Amongst the strategies for harvesting microalgae biomass, the coagulation–flocculation method is expedient. However, this method requires the use of coagulants that can interfere with the chemical composition and functionality of the biomass. Therefore, the need to establish the nexus between the harvesting coagulant and the biomass nutrient assemblage is germane. The physicochemical characteristics and the nutrient speciation of biomass harvested with either synthetic ($AlCl_3$ and $FeCl_3$) or naturally sourced (*Moringa oleifera*, gastropod shell and *Margaritarla discoidea*) coagulants were evaluated. The influence of the different coagulants on the forms and patterns of nutrients (*i.e.*, phosphorus, nitrogen and potassium) in the harvested biomass was evaluated. The physicochemical characteristics of the biomass, which influence nutrient availability, were impacted to various degrees by the different coagulants studied. The different coagulants used had no effect on the total phosphorus fraction of the harvested biomass, but impacted the available phosphorus fraction. All the coagulants investigated enhanced the available nitrogen in the biomass, as the percentage of the available nitrogen that constitute the total nitrogen fraction was >90% in all the harvested biomass samples, but less than 80% in the non-coagulant-biomass. None of the coagulants studied impacted the species of potassium in the biomass.

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Sustainability spotlight

Microalgae biomass is a viable resource for the production of renewable biofertilizer, but transforming the highly dispersed miniscule microalgae cells into harvestable biomass is challenging. Amongst the strategies for harvesting microalgae biomass, the coagulation–flocculation method is expedient. However, this method requires the use of chemical coagulants that can interfere with the chemical composition and functionality of the biomass. Premised on the use of microalgae biomass as a nutrient resource in sustainable agricultural practice and the possible incorporation of the coagulant fraction in the harvested biomass, we hereby established the nexus between the harvesting coagulant and the biomass nutrient assemblage.

1 Introduction

Consequent upon the need to ensure global food security through sustainable agricultural practice, the use of microalgae biomass is now being investigated as a green and sustainable resource substitute for conventional synthetic fertilizers and growth promoters.[1–5] This emerging interest in the use of the microalgae biomass is ascribed to its nutrient-rich status, and the potential for cultivation in nutrient-rich wastewater for the dual purposes of nutrient recovery and wastewater purification.[6] Microalgae biomass is endowed with both macro- and micronutrients that are crucial for crop development and growth, and are considered eco-friendly and probable substitutes for synthetic fertilizers and plant growth regulators.[7] This is because a wide range of bioactive compounds, including plant-growth-promoting substances, such as phytohormones (gibberellin, auxin, cytokinin, abscisic acid, and ethylene), phycobilins, amino acids, and carotenoids, are produced from biomass.[8–10] It has been reported that Cyanobacteria, microalgal species, take up the available phosphorus (P) species into the aqueous matrix and integrate them into the cell biomass. This cellular bound-P is then made available to plants by slow release,

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through autolysis and secretion, or decomposition of the dead algal cells.[11] It was posited that Cyanobacteria improve the bioavailability of P by solubilizing organic-P through phosphatase enzyme production.[11,12] Insoluble P species, such as $(Ca)_3(PO_4)_2$, $FePO_4$, $AlPO_4$, or $(Ca_5(PO_4)_3OH)$, in sediments and soils are solubilized by synthesizing chelators, which aids the release of P for plant uptake.[12]

Studies have shown that the use of *Scenedesmus subspicatus*, combined with humic acid, and *Spirulina platensis*, combined with cow dung, in separate experiments, improved the growth, yield, and content of pigments in the leaves of onions, and also elevated the levels of biochemicals and minerals in the crop.[13,14] The application of microalgae biomass improved the growth of tomatoes,[15–17] cucumbers,[15,18] eggplants,[19] peppers,[20] lettuce,[21,22] *etc.*

Considering the physicochemical characteristics (*e.g.*, microscopic nature and high surface charge density) of the microalgae cells, which inhibit aggregation in an aqueous system, harvesting the biomass for industrial and agricultural application is difficult. Since the harvested microalgae biomass is used in agriculture, directly, or indirectly after some valuable constituents have been extracted, the biomass constituents can impact the nutrient availability. Therefore, a careful choice of coagulant for harvesting microalgae biomass is apposite, because the constituents of the chosen coagulant are prone to altering the physicochemical characteristics of the biomass, impeding nutrient availability, and providing an avenue for the entry of undesirable biomass constituents into the food chain. It was posited that in the design of a sustainable phosphorus-recovery system, the choice of reactive metal species is vital.[23] This is because phosphorus that is strongly bound to the reactive metal species cannot be recovered for either industrial or agricultural usage. For example, Al^{3+} and Fe^{3+} show higher affinity for P capture, but their use for phosphorus recovery purposes is discouraged because the captured phosphorus is obstinately bound to the metal phase. Moreover, aluminum is noxious to many plants and some soil organisms.[24–26]

The major microalgae biomass harvesting methods comprise filtration, flotation, centrifugation, sedimentation, coagulation–flocculation, electrocoagulation, or hybrids of these processes.[27–30] Coagulation–flocculation is a simple harvesting method for bulk microalgae biomass production, but the coagulant of choice is bound to impact the physicochemical characteristics of the biomass produced.[31,32] The issue of aluminium salt causing cell lysis, where the outer membrane of the microalga is ruptured, subsequently releasing the desired intracellular compounds into the water, has been reported.[33] It has been observed that a significant difference occurred between the yield of biofuel produced from microalgae biomass harvested with ferric salt and that from a non-coagulant centrifugation method.[34] A difference in the biomass yield from microalgae harvested with aluminum salt and that harvested with cationic starch[35] has been documented. Aluminum salt was found to inhibit transesterification reactions and negatively impacted biodiesel production.[36] It was postulated that the inorganic fractions of the coagulant that is embedded in the harvested biomass caused an increase in ash content, thereby reducing the calorific value of the biomass.[36] These embedded inorganic fractions are also capable of serving as a catalyst, catalyst promoter, or catalyst support, that can promote thermochemical conversion or act as adsorbents for product cleanup.[37,38] Consequently, it was posited that the incorporation of chemical species during the coagulation–flocculation process and the implications for the end-use or products should be systematically interrogated.[39]

Premised on the use of microalgae biomass as a nutrient resource in sustainable agricultural practice and the possible incorporation of the coagulant fraction in the harvested biomass, it is hereby hypothesised that the coagulant fraction can tinker with the nutrient availability. In order to validate this hypothesis, the effects of both synthetic ($AlCl_3$ and $FeCl_3$) and naturally sourced (*M. oleifera* (MO) seed extract, gastropod shell and *M. discoidea* fruit seed extract (MDFE)) coagulants on the physicochemical characteristics (*i.e.*, pH value, electrical conductivity (EC) value, salt index (SI), and E3/E5 ratio), the elemental composition (*i.e.*, carbon, hydrogen, nitrogen, and sulfur), the surface functional groups, and nutrient (*i.e.*, N, P and K) speciation of the biomass harvested through different coagulant regimes were investigated.

2 Material and methods

2.1 Collection of eutrophicated water

The microalgae were sampled from a eutrophicated fish pond located at a village close to the university (Ayegunle-Akoko, latitude; 7° 20′ 43″ N, and longitude: 5° 41′ 39″ E). The microalgae composition has been previously identified and described.[40] The pH of the eutrophicated water was 7.2, the microalgae biomass concentration was 5.7 g L^{-1}, and the species of microalgae identified in the eutrophicated system included *Apatococcus lobatus*, *Arthronema africanum*, and *Aphanocapsa* sp.[40]

2.2 Determination of optimum coagulant dosage

The synthetic coagulants were prepared from both aluminium ($AlCl_3$) and ferric ($FeCl_3$) alum. Three different coagulants obtained from natural sources were prepared thus:

(i) MO: The seeds were obtained from dry pods collected from the university farm, pulverised in a laboratory grinding machine and stored in an air-tight plastic container pending usage. Premised on findings that the active coagulating ingredient of the MO seeds is best extracted in a solution of high ionic strength,[41,42] the extraction was carried out as follows: 5 g of the MO seed powder was dispersed in 100 mL of 1.0 M NaCl solution, and stirred on a magnetic stirrer for 30 min, at ambient tropical temperature, to extract the active coagulating component of the seed powder. The suspension was filtered using Whatman grade 1 filter paper, and the filtrate was used as the primary coagulant. The extraction of the active coagulant fraction of MO was carried out afresh for each use.

(ii) Gastropod shell (CGS): The coagulant from GS was prepared as previously reported.[43] The raw GS was subjected to thermal treatment in a furnace at 1000 °C for 2 h to obtain CGS. The coagulant suspension was prepared by the dispersion of 0.5 g of CGS in 10 mL of water.

(iii) MDFE: The fresh fruits were collected and the extraction of the coagulant fraction was carried out by adopting the procedure described in ref. 40 and 44. The coagulant was extracted by grinding 50 g of the fruits in 25 mL of distilled water, for a total period of 3 min. The suspension was filtered using a Whatman grade 1 filter paper, and the filtrate was used as the primary coagulant for microalgae harvesting. Extraction from the fresh fruit seed was carried out daily for the coagulation–flocculation experiment.

(iv) As a control, the microalgae biomass was harvested using a non-coagulant approach *via* centrifugation (CG), at 3000 *g* for 10 min.

The optimum dosage for the respective coagulants was determined in a fixed volume (500 mL) of the microalgae suspension placed in a 1 L beaker, at a fixed optical density of the microalgae solution. Varying dosages of the respective coagulant were added in each case: for $AlCl_3$ and $FeCl_3$, the dosage ranged between 25 and 100 mg L^{-1}, for MDFE, the dosage ranged between 1 and 8 mL, for MO, the dosage was 5 to 15 mL, and for CGS, the dosage was 0.3–1.6 g L^{-1}.

After the addition of the respective coagulant dosages, the mixture was stirred vigorously at 200 rpm for 2 min, and then slowly at 50 rpm for a period of 30 min. Subsequently, the mixture was allowed to settle for a period of 1 h, before the sample was withdrawn and the optical density (OD) value was determined at $\lambda_{max} = 680$ mm (*i.e.*, the λ_{max} value obtained from the spectra of UV/Visible spectrophotometry scanned between 400 and 800 nm) using a UV-Visible spectrophotometer.

The harvesting efficiency (HE%) was calculated using eqn (1):

$$\text{HE } (\%) = \frac{(\text{OD}_i - \text{OD}_f)}{(\text{OD}_i)} \times 100 \quad (1)$$

where OD_i is the optical density of the raw microalgae suspension, and OD_f is the value of the treated sample.

The coagulant dose with the highest HE (%) value represents the optimum coagulant dose.

2.3 Biomass harvesting

The microalgae biomass harvesting was carried out, using the respective optimum coagulant dosage, in a 15 L plastic container filled with 10 L of microalgae suspension. The solutions were stirred vigorously for 2 min and slowly for 30 min and allowed to settle for 60 min. The wet microalgae biomass was separated from the solution and dried in a laboratory oven at 70 °C. The control microalgae biomass sample, with no harvesting coagulant, was obtained by centrifugation at 10 000 rpm for 10 min.

2.4 Biomass characterization

The physicochemical characteristics (*i.e.*, pH value, EC, SI, and E3/E5 ratio) of the dried algae biomass (DAB) were determined using the methods described by Li *et al.*[45] The pH value was determined after 0.25 g of DAB was completely dispersed in 250 mL of deionised water in a 500 mL beaker, using a pre-calibrated pH meter. The EC of the biomass suspension was determined by accurately weighing 1 g of DAB into 400 mL of deionised water in a 500 mL beaker. The mixture was stirred for 10 min, and the EC was determined using an EC meter.

The SI value was determined by dispersing accurately weighed $NaNO_3$ (1 g) in 400 mL of deionised water in a 500 mL beaker and stirring for 10 min. An equal weight (1 g) of DAB was also dispersed in 400 mL of deionised water and vigorously stirred, before the EC value was determined. The SI value was calculated using eqn (2):

$$\text{SI} = \frac{\text{EC of 1 g dried microalgae biomass}}{\text{EC of 1 g NaNO}_3\text{ solution}} \times 100 \quad (2)$$

The UV-VIS absorbance ratio between $\lambda_{max} = 350$ nm and 550 nm (E3/E5) of the DAB was determined by adding 0.2 g of the biomass into 10 mL of 0.05 N $NaHCO_3$. Thereafter, the absorbance of the mixture was taken at 350 nm and 550 nm, respectively, using a UV-Visible spectrophotometer. E3/E5 was calculated by dividing the absorbance value at 350 nm by that at 550 nm.

The surface functional groups on the DAB were determined using Fourier-transform infrared spectroscopy (FTIR). The DAB (1.5 mg) was ground with 300 mg of KBr and vacuum compressed to form a testing disc. The FTIR spectra were recorded on an FTIR spectrophotometer (PerkinElmer Spectrum 100 with ATR unit), with a resolution of 2 cm^{-1}, for a wavenumber range of 4000 to 400 cm^{-1}, after 64 scans. The elemental composition (wt% oven-dry weight) (*i.e.*, CHNS) was determined for the DAB, using an elemental analyzer (Vario MACRO cube, Germany).

2.5 Nutrient speciation in biomass

2.5.1 Phosphorus speciation in biomass. The standard measurement and harmonised testing procedure for P fractionation in different biowastes was used (Fig. 1a).[46,47] The P species determined included total P (TP), soluble P (SP), which is the P weakly bound to the sample matrix, Fe/Al mineral adsorbed P (AP), which is the moderately labile P, exchangeable P (EP) and insoluble P (ISP), which are the stable forms of P.

In order to determine the TP fraction in the DAB, 0.50 g of DAB was calcined for 3 hours at 450 °C, before 20 mL of 3.5 M HCl was added to the cooled ash and then agitated for 16 hours. The TP value was determined after the mixture was centrifuged for 15 min and filtered. The P fractions present in 0.5 g of DAB were sequentially extracted by water, 0.5 mol L^{-1} $NaHCO_3$, 0.1 mol L^{-1} NaOH and 1.0 mol L^{-1} HCl, 30% H_2O_2 with H_2SO_4 to obtain the SP, EP, AP, ISP, and residual P (RP). For each extraction, the mixture was oscillated at 120 rpm for 16 h, before the mixture was centrifuged and then filtered with a 0.45 μm filter, as presented in Fig. 1. In each case, the different P fraction was determined by digesting the filtrate with potassium persulfate ($K_2S_2O_8$), and then the concentration of P was determined by the molybdenum-blue ascorbic acid method with a UV-VIS spectrophotometer.[48]

The Ca-bound P species fractionating protocol, initially proposed by Chang and Jackson,[49] and subsequently modified

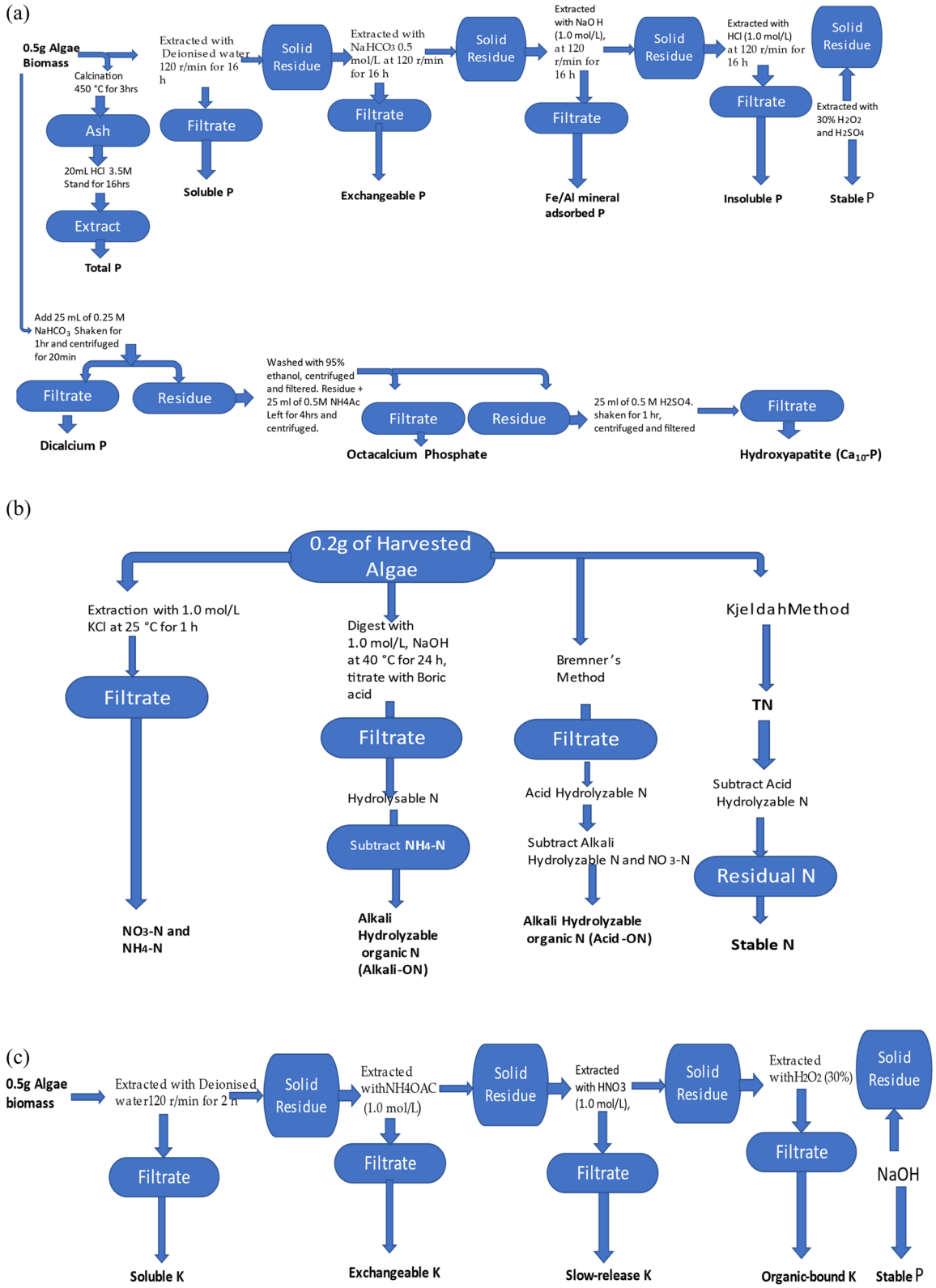

Fig. 1 (a) Determination of P speciation in DAB. (b) Determination of N speciation in DAB. (c) Determination of K speciation in DAB.

by Gu and Jiang,[50] was adopted for the determination of Ca-bound P speciation. This is a method that is used to fractionate P in calcareous soil, which includes different operationally defined phosphorus fractions. 0.50 g of accurately weighed DAB was added to 50 mL polyethylene centrifuge tubes holding 25 mL of 0.25 M $NaHCO_3$ (pH 7.5). The mixture was

agitated on an orbital shaker at 25 °C for 1 h and centrifuged at 5000*g* for 20 min. The filtrate was analysed for dicalcium phosphate (Ca_2-P). The residue was washed twice with 95% ethanol (12.5 mL for each wash), the mixture was centrifuged and the filtrate discarded before 25 mL of 0.5 M NH_4Ac (pH 4.2) was added. The mixture was left to stand for 4 h, to enable complete dispersion of the residue. The evenly dispersed mixture was agitated for 1 h at 25 °C, followed by centrifugation at 5000*g* for 20 min, and the filtrate was analysed for octacalcium phosphate (Ca_8-P). Ten-calcium phosphate (Ca_{10}-P) was determined by adding 25 mL of 0.5 M H_2SO_4 to the residue from the determination of Ca_8-P. The mixture was agitated for 1 h at 25 °C, followed by centrifugation and filtration, before analysis was carried out for phosphate, as Ca_{10}-P.

2.5.2 Nitrogen speciation in biomass. The concentration of total nitrogen (TN) in the DAB was determined by the standard Kjeldahl method.[48] The other nitrogen species determined included inorganic N (primarily NO_3–N and NH_4–N), organic N, and non-hydrolyzable nitrogen (*i.e.*, residual N) (Fig. 1b). The concentrations of NO_3–N and NH_4–N (inorganic N) in the DAB were determined by extracting 0.2 g of each DAB sample with 1.0 mol L^{-1} KCl at 25 °C for 1 h. The concentrations of NO_3–N and NH_4–N in the filtrate were determined using the recommended standard method.[48] The concentration of alkali-hydrolyzable N in each DAB was determined through NaOH hydrolyzation diffusion (1.0 mol L^{-1}, at 40 °C for 24 h) and subsequent titration with boric acid.[51] The concentration of alkali-hydrolyzable organic N (alkali-ON) was obtained by deducting the value of NO_3–N from acid-hydrolyzable N.

The concentration of acid-hydrolyzable N in each DAB sample was measured according to the Bremner method: exactly 0.2 g of DAB was extracted with 6 mol L^{-1} hydrochloric acid, followed by digestion with sulfuric acid and a catalyst (K_2SO_4–$CuSO_4$–Se powder). The value of the acid-hydrolyzable N was finally obtained with distilled/semi-micro titration.[52] The concentration of residual N (RN) was obtained by subtracting the value for the acid-hydrolyzable N from the TN value.

2.5.3 Potassium speciation in biomass. The K speciation determined included soluble K (SK), exchangeable K (EK), slow-release K (SRK), organic-bound K (OBK), and residual K (RK) (Fig. 1c). The extraction was conducted using water, ammonium acetate, nitric acid, and hydrogen peroxide, in turn, to obtain SK, EK, SRK, and OBK from 0.5 g of DAB, respectively.[53,54] The mixture oscillated at 120 rpm for 2 h during each extraction process. The mixture was centrifuged and then filtered using a 0.45 μm membrane after each extraction process. The concentration of K in each filtrate was determined using a flame photometer.

2.6 Process and measurement reproducibility

For microalgae harvesting using different coagulants, a grab sample of the eutrophicated water was collected from the pond to form a single system from which all the experiments were conducted. The physicochemical characterization of biomass and the determination of the harvested biomass nutrient profiles were conducted in triplicate. All data obtained were subjected to one-way ANOVA. Statistical means were separated with the new Duncan's multiple range test at 95% level of significance using the Statistical Package for Social Sciences (SPSS) software, version 24.0.

3 Results and discussion

3.1 Microalgae biomass harvesting

Maximum harvesting efficiencies of 96.5%, 96.3%, 95.9%, 94.8%, and 91.7% were achieved using 25 mg L^{-1} $AlCl_3$, 50 mg L^{-1} $FeCl_3$, 0.8 g L^{-1} CGS, 16 mL L^{-1} MDFE and 12 mL L^{-1} MO, respectively. The results obtained in this study were similar to those achieved by Wang *et al.*,[55] with 97% harvesting efficiency of *Coccomyxa* sp. and *Chlorella vulgaris* using 30 mg L^{-1} $AlCl_3$. The optimum dosage of $FeCl_3$ (50 mg L^{-1}) obtained in the present study was much lower than that employed for some freshwater microalgal strains (150 mg L^{-1} $FeCl_3$).[56] The observed gap in the optimum coagulant dosage between our microalgae sample and those reported in the literature could be attributed to the difference in the physicochemical characteristics, which have a great influence on the coagulation efficiency. It was reported that the alkalinity/pH of a medium affects the hydrochemistry of the coagulants, predominantly speciation transformation, as well as the distribution of the coagulants after dosing.[57] The pH of the raw microalgae sample used in this study was near neutral (pH value = 7.2), and required a rather low $FeCl_3$ dosage for favourable coagulation. Furthermore, the coagulation efficiency of different coagulants can be affected by microalgae species, cell sizes, cell surface features, cell densities, zeta potentials, and cell stability.[55,58,59] It has been reported that 30 mg L^{-1} $AlCl_3$ harvested 97% of both *Coccomyxa* sp. K.J. and *Chlorella vulgaris*; however, 1000 mg L^{-1} $AlCl_3$ was required to achieve the same harvesting efficiency (%) for *Chlamydomonas reinhardtii*.[55]

3.2 Physicochemical characteristics of harvested biomass

The dry weight percentages of CHNS in the different DAB samples are presented in Table 1. All the DAB contained CHN in various proportions, but sulfur was undetectable (Table 1). The carbon content (%) in the various DAB varied, and CG-DAB (43.42%), MO-DAB (43.02%) and MDFE-DAB (35.52%) gave the highest percentage carbon content amongst the coagulants studied. The presence of sulfur in harvested microalgae biomass has either been reported at relatively low concentrations (<1%),[34,43,60,61] or has been undetectable.[40,62]

As expected, the natural-based coagulants gave biomass with the highest carbon content, because of the contribution from the carbonaceous fraction of the coagulant. This observation aligned with the report of Soares *et al.*[62] that showed that a tannin-based polymer produced biomass with relatively higher carbon content (47.7%) than the other inorganic-based coagulants investigated. The higher carbon content biomass harvested with a natural coagulant could also be ascribed to the effectiveness of these coagulants in the removal of the carbonaceous fraction of the eutrophicated water.

Table 1 The percentage CHNS composition of the biomass harvested with different coagulants[b]

Element (%)	MO-DAB	MDFE-DAB	$AlCl_3$-DAB	$FeCl_3$-DAB	CGS-DAB	CG-DAB
Carbon	43.1	35.5	30.3	30.9	26.1	43.4
Hydrogen	6.8	5.6	5.2	5.0	3.2	6.7
Nitrogen	6.4	4.2	6.3	6.8	4.4	9.8
Sulfur	UND[a]	UND[a]	UND[a]	UND[a]	UND[a]	UND[a]

[a] UND = undetectable. [b] N.B: MO-DAB (MO-harvested DAB), MDFE-DAB (MDFE-harvested DAB), $AlCl_3$-DAB (aluminium-chloride-harvested DAB), $FeCl_3$-DAB (iron-chloride-harvested DAB), CGS-DAB (CGS-harvested DAB), CG-DAB (centrifuged DAB).

Carbon is indisputably regarded as the most important regulator of soil fertility, with an impact on a wide range of soil parameters that boost crop performance. Organic carbon influences soil physicochemical performance by promoting healthy soil structure, which improves tilth, porosity, and water-holding capacity. The high percentage of carbon content in the harvested DAB showed that they have potential applications as biofertilizers, to boost and enhance soil productivity. All the DAB samples contained an appreciable amount of N (range = 9.8–4.2%) (Table 1).

The C/N ratio (%) calculated for the different DAB was 6.8 for MO-DAB, 8.6 for MDFE-DAB, 4.8 for $AlCl_3$-DAB, 4.5 for $FeCl_3$-DAB, 5.9 for CGS-DAB, and 4.4 for CG-DAB. The ratio of the carbon and nitrogen (C/N) constituents of biomass has been identified as a salient parameter that determines the rate of biochemical conversion of biomass in soil. Therefore, biomass with a C/N ratio > 25 is not easily degraded by microbial action.[32] Furthermore, the C/N ratio of commercial organic amendment must be <30 to enhance nitrogen availability to plants. For the respective DAB, the C/N ratios ranged between 8.6 and 4.4, which is comparable with the values reported (ranging between 3 and 7) for other biomass-based fertilizers.[32,63,64]

The EC values of the different coagulant-DAB samples were investigated to evaluate the degree of dissolution of ions from the DAB and the nutrient availability potentials (Table 2). The EC values obtained ranged between 305.7 and 489.4 μs cm^{-1}. The highest value of EC (489.4 μs cm^{-1}) was obtained for $FeCl_3$-DAB. The observed value can be ascribed to the relatively high concentration of $FeCl_3$ (50 mg L^{-1}) (*i.e.*, the required optimum coagulant dosage) used for the harvesting process, which dissolved in the aqueous matrix and enhanced the ionic activity of the medium. The EC values of $AlCl_3$-DAB (447 μs cm^{-1}), MO-DAB (402.4 μs cm^{-1}) and MDFE-DAB (412.5 μs cm^{-1}) were also relatively high. CG gave the lowest EC value (305.7 μs cm^{-1}).

The soil pH value significantly affects nutrient availability for plant uptake. Nutrients such as N, P, K, are mostly available within the neutral and alkaline pH value range of 6.5 to 8. The pH value range (*i.e.*, 6.4–7.9) of the different harvested microalgae was within the desirable neutral–alkaline range that promotes nutrient availability (Table 2). MDFE-DAB gave the lowest pH value of 6.4, while CGS-DAB gave the highest pH value of 7.9. The pH value exhibited by each of the DAB is attributed to the influence of each coagulant used for harvesting.

The SI value of a fertilizer is the degree of salt concentration that the fertilizer produces in a soil solution.[65] It is also defined as a proportion of the increase in osmotic pressure of the salt solution produced by the fertilizer to the osmotic pressure of the same weight of sodium nitrate ($NaNO_3$).[66] The SI values obtained were higher for $FeCl_3$-DAB (46.7), $AlCl_3$-DAB (40.5) and CGS-DAB (40.3), than for MO-DAB (28.2) or MDFE-DAB (25.4) (Table 2). The relatively higher values of SI in $FeCl_3$-DAB, $AlCl_3$-DAB and CGS-DAB were attributed to the chemical profiles of each coagulant. The SI values obtained were comparable with the values obtained for the solid residue from the ammonium sulfite process (58.4) and Kraft pulping process (39.1).[32] The values were also comparable with commercial fertilizers such as ammonium polyphosphate (20.0), MAP (26.7), DAP (29.2), and ammonia (47.1).[67] Fertilizers with high SI is an indication of a high concentration of soluble salts, which may not necessarily be soluble nutrient fractions. As soon as these salts dissolve in the soil, they cause an upsurge in the salt concentration of the soil solution and subsequently increase the solution's osmotic potential. The higher the osmotic potential of the soil solution, the more difficult it is for plant seeds or plants to extract the soil water required for growth.

A UV-VIS absorbance ratio between 350 nm and 550 nm is a pointer to the degree of humification and the relative molecular size of organic fertilizer.[32] The value of E3/E5 obtained for the different DAB samples ranged between 1.3 and 1.9 (Table 2). A high E3/E5 ratio shows that the material has a small molecular size and a low content of condensed aromatic rings,[68] while a low value indicates the presence of a high content of aromatic rings. The E3/E5 values obtained for the different harvested

Table 2 Effects of harvesting coagulant on the physicochemical characteristics of DAB

Parameter	MO-DAB	MDFE-DAB	$AlCl_3$-DAB	$FeCl_3$-DAB	CG-DAB	CGS-DAB
EC (μs cm^{-1})	402.4 ± 1.2	412.5 ± 1.5	447.0 ± 0.9	489.4 ± 1.6	305.7 ± 1.4	387.5 ± 2.1
pH	7.4 ± 0.5	6.4 ± 0.7	6.6 ± 0.2	6.4 ± 0.3	7.2 ± 0.3	7.9 ± 0.2
SI	28.2 ± 0.45	25.4 ± 0.27	40.5 ± 0.11	46.7 ± 1.1	20.3 ± 0.91	40.2 ± 0.23
E3/E5	1.9 ± 0.1	1.7 ± 0.1	1.3 ± 0.2	1.3 ± 0.4	1.4 ± 0.1	1.6 ± 0.3

algae are low (range = 1.3–1.9), compared with the E3/E5 values obtained for residues from the ammonium sulfite process (13.9) or Kraft pulping process (7.2).[32] The E3/E5 values obtained for MO-DAB (1.9) and MDFE-DAB (1.7) were higher than those obtained for the other coagulants, but the values were far smaller than the E3/E5 values for humic acid (6.0) or fulvic acid (8.0) obtained by.[68] This suggests that MO-DAB and MDFE-DAB have small molecular sizes, followed by DAB-CGS (1.6) but not as small as the molecular size of humic acid. Fertilizers with small molecules are absorbed more easily through plant roots, stems, and leaves than fertilizers with larger molecules.[69] With reference to the E3/E5 values obtained in this study, nutrient fraction absorption from the biomass by a plant will decrease in the order MO-DAB > MDFE-DAB > CGS-DAB > CG-DAB > $FeCl_3$-DAB > $AlCl_3$-DAB.

The FTIR spectra of the different coagulant-DAB samples (Fig. 2) showed different peaks that revealed the presence of protein and polysaccharides. Apart from the difference in the peak intensities, the peak positions shown by both the synthetic and non-synthetic harvested DAB were similar. The % transmittance values of the diagnostic peaks in the coagulant-DBA samples were lower than that of CG-DBA, which made the peaks appear to be more prominent in coagulant-DBA than in CG-DBA. The FTIR spectra of all the DAB showed broad peaks, with different intensities, at 3300–3200 cm^{-1}, which has been attributed to the stretching vibrations of N–H and OH.[10,70] The two sharp peaks between 2900 and 2800 cm^{-1}, which are more prominent in the MO-DAB spectra, are assigned to C–H stretching. The occurrence of a relatively strong absorption around 1600 cm^{-1} present in all the samples is attributed to the characteristic peak of the carboxylic group.[71] The prominent peaks that appeared between 1650 and 1580 cm^{-1} were assigned to N–H stretching[72] and the 1500−1400 cm^{-1} peaks were characteristics of C–C stretching in aromatic rings. The peak at 1446–1372 cm^{-1} present in all the coagulant-DAB samples is attributed to –OH distortion and C–O stretching of phenolic OH. The sharp peaks between 1200 and 1000 cm^{-1} were assigned to the presence of C–O–C and C–O functional groups present in all the DAB. The presence of Cyanobacteria (*Aphanocapsa* sp.) in the microalgae colony was attributed to the P=O asymmetric vibration peaks that appeared around 1246 cm^{-1}.

3.3 Phosphorus speciation in biomass

The forms and patterns of the phosphorus species in the different coagulant-DAB samples are presented in Table 3. The values of the total phosphorus (TP) for the different coagulant-DAB samples varies significantly ($p \leq 0.05$) as follows: MDFE-DAB (5457.4 ± 27.4 mg kg^{-1}) > MO-DAB (5360.8 ± 17.3 mg kg^{-1}) = $AlCl_3$-DAB (5301.7 ± 15.6 mg kg^{-1}) > $FeCl_3$-DAB (5227.1 ± 15.8 mg kg^{-1}) = CGS-DAB (5210.4 ± 18.9 mg kg^{-1}) > CG-DAB (5072.4 ± 22.0 mg kg^{-1}). The TP contents in the different DAB were higher than the values detected in *Chlorella vulgaris* (2500–4000 mg kg^{-1}) cultivated in municipal wastewater,[73] but lower than the TP content of harvested microalgae (8000–20 000 mg kg^{-1}) grown in synthetic wastewater.[74] Studies have shown that microalgae adjust the N and P concentrations in their system with the nutrient concentration in the surrounding water.[75,76] Although the biomass P accumulation is influenced by the external supply of P and N, N accumulation is independent of P.[75,76] This is related to the fact that nitrogen is used primarily for protein synthesis, while P is incorporated into ribosomal RNA.[75]

The concentrations of soluble P in MDFE-DAB (1498.1 ± 27.1 mg kg^{-1}) and CG-DAB (1470.5 ± 10.1 mg kg^{-1}) were not significantly different from each other, but were significantly higher than the DAB harvested with metal salts, $AlCl_3$ (704.3 ± 4.6) and $FeCl_3$ (733.3 ± 7.6) (Table 3). The soluble P obtained in $FeCl_3$-DAB, $AlCl_3$-DAB and CGS-DAB were equivalent to 14%,

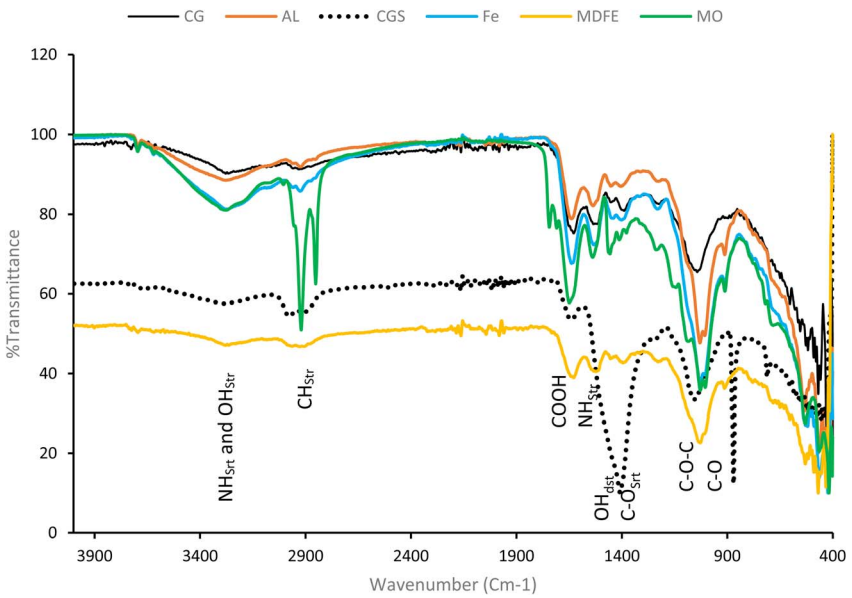

Fig. 2 A comparison of the surface functional group of the coagulant-DAB samples.

Table 3 Phosphorus (P) species in DAB from different coagulants[a]

	Soluble P	Exchangeable P	Fe/Al-adsorbed P	Residual P	Insoluble P	Total P
$AlCl_3$	704.3 ± 4.6[b]	800.2 ± 7.6[a]	1877.1 ± 8.5[f]	881.90 ± 5.2[c,d]	1038.2 ± 10.5[c]	5301.6 ± 15.6[c]
$FeCl_3$	733.3 ± 7.6[b]	784.9 ± 6.5[a]	1705.6 ± 7.8[e]	654.96 ± 3.3[b]	1348.3 ± 8.7[e]	5227.1 ± 15.8[b]
CGS	609.2 ± 8.7[a]	2179.8 ± 7.9[e]	432.9 ± 7.3[a]	869.99 ± 6.8[c]	1118.6 ± 12.3[d]	5210.4 ± 18.9[b]
MDFE	1498.1 ± 27.2[d]	1693.9 ± 7.1[d]	524.5 ± 5.1[b]	897.89 ± 7.5[d]	843.0 ± 4.5[b]	5457.4 ± 27.4[d]
MO	1408.4 ± 11.4[c]	1529.9 ± 7.3[c]	750.2 ± 5.3[d]	877.60 ± 10.0[c,d]	794.6 ± 5.3[a]	5360.8 ± 17.3[c]
CG	1470.5 ± 10.1[d]	1115.5 ± 9.8[b]	550.4 ± 4.7[c]	525.56 ± 6.9[a]	1410.5 ± 9.9[f]	5072.4 ± 22.0[a]

[a] Values are mean ± standard error for 3 replicates. Means with the same letters on the same column are not significantly different from each other at $p \geq 0.05$ (Duncan's multiple range test).

13.3% and 11.6% of their TP, respectively, while the soluble P in MDFE-DAB and MO-DAB represent 27.4% and 26.3% of their total phosphorus, respectively. The relatively lower amount of soluble P recorded in the DAB harvested with metal ions and CGS was attributed to the formation of insoluble Al–P, Fe–P and Ca–P species. Phasey *et al.*[77] suggested that conventional coagulants (*i.e.*, iron and aluminium salts) contaminate the harvested biomass and lock up the embedded phosphorus, thereby preventing their beneficial reuse as biofertilizer. Saliu *et al.*[40] confirmed the formation of hydroxyapatite, a less soluble Ca–P species, when thermally treated GS was used for P recovery from human urine. The higher soluble P obtained in the plant-based-DAB samples showed that the P fraction will be readily available for plant uptake but may suffer nutrient loss through run-off.

Significantly high values of insoluble phosphorus were obtained from CG-DAB (1410.5 ± 9.9 mg kg^{-1}), $FeCl_3$-DAB (1348.3 ± 8.7 mg kg^{-1}) and CGS-DAB (1118.6 ± 12.3 mg kg^{-1}) compared to the significantly lower values of MDFE-DAB (842.9 ± 4.5 mg kg^{-1}) and MO-DAB (794.6 ± 5.3 mg kg^{-1}). Significantly high values of Fe/Al-adsorbed P were recorded in $AlCl_3$-DAB (1877.1 ± 8.5 mg kg^{-1}) and $FeCl_3$-DAB (1705.6 ± 7.8 mg kg^{-1}), compared with MO-DAB (750.2 3 mg kg^{-1}), MDFE-DAB (524.5 ± 5.1 mg kg^{-1}), CGS-DAB (432.9 ± 7.3 mg kg^{-1}) and the centrifuged-DAB (550.4 ± 4.7 mg kg^{-1}) (Table 3). Studies have shown that P availability for plant uptake decreases when P is bound to Fe and Al.[78,79]

The determination of Ca–P species in DAB-CGS (Table 4) showed the prevalence of Ca_{10}-P (*i.e.*, hydroxyapatite), which further confirmed the higher insoluble P fraction. Dicalcium P, a more soluble species, was significantly higher in CGS-DAB (619.3 ± 14.5 mg kg^{-1}), compared with MDFE-DAB (140.3 ± 12.9 mg kg^{-1}), MO-DAB (102.3 ± 4.9 mg kg^{-1}) or $AlCl_3$-DAB (134.2 mg kg^{-1}). Dicalcium-P, being a more soluble P species, is an indication that the P fraction will be readily available for plant growth.

3.4 Nitrogen speciation in biomass

The N species present in the different coagulant-DAB samples are presented in Table 5. The TN fraction for the harvested DAB varies significantly ($p \leq 0.05$) as follows; 111 633 ± 47.9 mg kg^{-1} (MDFE-DAB) > 96 594 ± 45.6 mg kg^{-1} (MO-DAB) > 81 971 ± 47.9 mg kg^{-1} ($FeCl_3$-DAB) > 81 799 ± 46.8 mg kg^{-1} (CG-DAB) = 81 754 ± 1.7 mg kg^{-1} (CGS-DAB) > 81 179 ± 52.0 mg kg^{-1} ($AlCl_3$-DAB). The TN contents of the different DAB were within the range of TN for the biomass of algae grown in municipal wastewater (20 500–90 000 mg kg^{-1}),[73] but higher than those reported for microalgae biomass from horticultural wastewater (60 800 mg kg^{-1})[80] and 70 190 mg kg^{-1}.[81] The nutrient concentration of the culture medium could be ascribed to the variations in the TN values observed. This is a pointer to the fact that a microalgae culture medium with higher concentration of TN has the potential to produce microalgae biomass with a higher TN fraction.

Inorganic nitrogen, such as NH_4–N and NO_3–N, may be readily absorbed and utilized by plants, and it is often used as nitrogen fertilizer because of the high N availability.[47] The highest inorganic N fraction (*i.e.*, NO_3 plus NH_4) was detected in MDFE-DAB and MO-DAB (66 008 mg kg^{-1} and 54 309 mg kg^{-1}, respectively), which accounted for 59.3% and 56.2% of the TN, respectively, than in CGS-DAB (54 176 mg kg^{-1}), $FeCl_3$-DAB (53 693 mg kg^{-1}), or $AlCl_3$-DAB (50 537 mg kg^{-1}), which accounted for 66.3%, 65.5%, and 62.3% of TN, respectively. Although the magnitude of the readily available N is higher in the plant-based-DAB than in the other coagulant-DAB samples, the relative percentage to TN was higher in the other coagulant-DAB samples than in the plant-based-DAB samples. The non-coagulant approach (*i.e.*, CG-DAB) gave the lowest magnitude of available N (40 972 mg kg^{-1}) and the relative percentage of inorganic-N to TN (50.1%). The high inorganic-N fraction of the coagulant-DAB samples showed that a high percentage of N fraction of the DAB will be readily available for plant nutrition.[82]

Simple organic nitrogen, such as amino acids, amides, and highly hydrolyzable protein nitrogen are classified as alkali-hydrolyzed organic nitrogen (alkali-ON), which can be directly and readily absorbed and utilized by plants, much like

Table 4 Ca–P species in DAB from different coagulants[a]

Sample	Dicalcium P	Ca_8-P	Ca_{10}-P
$AlCl_3$	134.2 ± 7.6[a,b]	103.9 ± 6.8[b]	166.0 ± 4.0[d]
$FeCl_3$	280.3 ± 13.4[c]	260.9 ± 6.7[c]	140.8 ± 4.7[c]
CGS	619.3 ± 14.5[d]	477.7 ± 10.0[d]	986.7 ± 6.7[e]
MDFE	140.3 ± 12.9[b]	120.0 ± 5.0[b]	73.2 ± 3.8[b]
MO	102.3 ± 4.9[a]	58.3 ± 3.8[a]	66.5 ± 3.3[a,b]
CG	105.4 ± 7.2[a]	74.6 ± 3.0[a]	55.2 ± 1.9[a]

[a] Values are mean ± standard error for 3 replicates. Means with the same letters on the same column are not significantly different from each other at $p \geq 0.05$ (Duncan's multiple range test).

Table 5 Nitrogen species in the DAB from different coagulants[a]

Sample	NO_3	NH_4	Alkali-ON	Acid-ON	RN	TN
$AlCl_3$	21 761.0 ± 9.2^c	28 776.0 ± 16.7^c	19 701.0 ± 9.8^a	3977.0 ± 5.2^b	6964.0 ± 11.0^e	81 179.0 ± 52.0^a
$FeCl_3$	22 789.0 ± 16.7^d	30 904.0 ± 12.1^d	21 506.0 ± 7.5^c	4151.0 ± 4.6^d	2621.0 ± 6.9^c	81 971.0 ± 47.9^c
GS	17 058.0 ± 6.4^b	37 118.0 ± 9.8^e	20 699.0 ± 7.5^b	4017.0 ± 7.5^c	2862.0 ± 5.2^d	81 754.0 ± 1.7^b
MD	28 295.0 ± 16.2^f	37 713.0 ± 6.4^f	40 302.0 ± 13.3^f	3916.0 ± 8.7^a	1407.0 ± 3.5^b	111 633.0 ± 47.9^e
MO	27 014.0 ± 13.9^e	27 295.0 ± 9.8^b	36 155.0 ± 8.7^e	4751.0 ± 6.9^e	1379.0 ± 6.4^a	96 594.0 ± 45.6^d
CG	15 067.00 ± 11.0^a	25 905.0 ± 8.1^a	22 732.0 ± 9.8^d	8573.0 ± 8.1^f	9522.0 ± 9.8^f	81 799.0 ± 46.8^b

[a] Values are mean ± standard error for 3 replicates. Means with the same letters on the same column are not significantly different from each other at $p \geq 0.05$ (Duncan's multiple range test).

inorganic nitrogen. The percentage of alkali-ON in the TN fraction of the different DAB samples was 37.4% (MO-DAB), 36.1% (MDFE-DAB), 27.8% (CG-DAB), 26.3% ($FeCl_3$-DAB), 25.3% (CGS-DAB), and 24.3% ($AlCl_3$-DAB). Significantly ($p \leq 0.05$) higher Alkali-ON contents were recorded for MDFE-DAB (40 302.0 ± 13.3 mg kg^{-1}) and MO-DAB (36 155.0 ± 8.7 mg kg^{-1}) (Table 5) due to the fact that they are plant-based coagulants, which have the capacity to incorporate more protein and organic nitrogen into the algae biomass.

Hot hydrochloric acid extracted nitrogen, such as hexosamine nitrogen and some heterocyclic nitrogen are referred to as acid-hydrolyzed organic nitrogen (acid-ON), while residual nitrogen (RN), exists in heterocyclic form and is bonded to heterocyclic or aromatic rings with a high degree of condensation. These nitrogen species are not readily available for plant uptake and are called slow-release nitrogen. The content of acid-ON (8573.0 ± 8.1 mg kg^{-1}) and RN (9522.0 ± 9.8 mg kg^{-1}) in the control (GC-DAB) were significantly higher than in all the other coagulant DABs (Table 5). Although the acid-ON of MO-DAB (4751.0 ± 6.9 mg kg^{-1}) was significantly lower, when compared with the control, it was higher than the other coagulant-DABs. MO-DAB was complemented by a significantly low value of RN (1379.0 ± 6.4 mg kg^{-1}). Significantly ($p \leq 0.05$) low acid-ON (3916.0 ± 8.7 mg kg^{-1}) and RN (1407.0 ± 3.5 mg kg^{-1}) contents were obtained in MDFE-DAB. In summary, the percentage of the sum of accessible nitrogen (*i.e.*, alkali-ON, nitrate N and ammonia N) in the TN content of MDFE-DAB (95.2%), MO-DAB (93.7%), $FeCl_3$-DAB (91.7%), CGS-DAB (91.6%), and $AlCl_3$-DAB (86.5%) were higher than for the non-coagulant approach (*i.e.*, CG-DAB (77.8%)). The unavailable nitrogen (acid-ON and RN) in the TN fraction of MDFE-DAB (4.8%), MO-DAB (6.3%), $FeCl_3$-DAB (8.3%), CGS-DAB (8.4%), and $AlCl_3$-DAB (13.3%) were much lower than for CG-DAB (22.2%).

3.5 Potassium speciation in biomass

The different forms of K present in the coagulant-DAB samples vary significantly, as presented in Table 6, such that MDFE-DAB (7895 ± 38.68 mg kg^{-1}) > MO-DAB (7495 ± 34.1 mg kg^{-1}) > CGS-DAB (5760.0 ± 44.5 mg kg^{-1}) > $FeCl_3$-DAB (5647.0 ± 30.0 mg kg^{-1}) = $AlCl_3$-DAB (5569.0 ± 33.5 mg kg^{-1}) = CG-DAB (5535.0 ± 31.8 mg kg^{-1}) (Table 5). The TK fractions of the DAB samples were within the range of the TK contained in algae grown in dairy effluent (6100–8800 mg kg^{-1})[83] but higher than those recorded for *Chlorella vulgaris* (4500 mg kg^{-1}) cultivated through inoculation of axenic microalgal cultures in appropriate growth media.[81]

Both the soluble K (SK) and exchangeable K (EK) are referred to as available potassium, which can be directly absorbed and utilized for plant growth. The sums of the available K (SK plus EK) in the DAB samples were 2981 mg kg^{-1} ($AlCl_3$-DAB), 2220 mg kg^{-1} ($FeCl_3$-DAB), 2242 mg kg^{-1} (CGS-DAB), 3575 mg kg^{-1} (MDFE-DAB), 3772 mg kg^{-1} (MO-DAB), and 2007 mg kg^{-1} (CG), which accounted for 53.5% ($AlCl_3$-DAB), 39.3% ($FeCl_3$-DAB), 38.9% (CGS-DAB), 45.3% (MDFE-DAB), 50.3% (MO-DAB) and 36.3% (CG-DAB) of TK.

HNO_3-extractable potassium, which is slow release K (SRK), and organic-bound K (OBK), the species of K that can be gradually converted to effective K for plant uptake, were also quantified. The percentage of the sum of these K species (SRK + OBK) in the TK fraction of the DAB samples are 26.8% ($AlCl_3$-DAB), 34.8% ($FeCl_3$-DAB), 42.1% (CGS-DAB), 45.0% (MDFE-DAB), 43.8% (MO-DAB) and 40.8% (CG-DAB).

The total percentage of available K species for plant uptake (SK + EK + SRK + OBK) were 80.3% ($AlCl_3$-DAB), 74.1% ($FeCl_3$-DAB), 81.0% (CGS-DAB), 90.3% (MDFE-DAB), 94.1% (MO-DAB) and 77.1% (CG-DAB). These available K species were much higher than the percentages of unavailable K (RK), which were

Table 6 Species of K in DAB from different coagulants

Sample	Soluble K (SK)	Exchangeable K (EK)	Slow-release K (SRK)	Organic-bound K (OBK)	Residual K (RK)	Total K (TK)
$AlCl_3$	1624.0 ± 5.2^d	1357.0 ± 7.5^d	512.0 ± 5.2^a	978.0 ± 8.1^b	1098.0 ± 7.5^c	5569.0 ± 33.5^a
$FeCl_3$	1128.0 ± 4.6^a	1092.0 ± 4.6^c	890.0 ± 6.9^b	1073.0 ± 6.4^c	1464.0 ± 7.5^e	5647.0 ± 30.0^a
GS	1513.0 ± 6.4^c	729.0 ± 2.9^a	1200.0 ± 11.0^c	1225.0 ± 13.3^d	1093.0 ± 11.0^c	5760.0 ± 44.5^b
MD	2018.0 ± 7.5^e	1557.0 ± 6.9^e	1673.0 ± 9.2^f	1882.0 ± 10.4^f	765.0 ± 4.6^b	7895.0 ± 38.7^d
MO	2191.0 ± 10.4^f	1581.0 ± 5.2^f	1445.0 ± 4.6^d	1839.0 ± 9.8^e	439.0 ± 4.0^a	7495.0 ± 34.0^c
CG	1256.0 ± 9.2^b	751.0 ± 4.0^b	1562.0 ± 9.8^e	699.0 ± 2.3^a	1267.0 ± 6.4^d	5535.0 ± 31.8^a

19.7% ($AlCl_3$-DAB). 25.9% ($FeCl_3$-DAB), 18.9% (CGS-DAB), 9.7% (MDFE-DAB), 5.9% (MO-DAB) and 22.9% (CG-DAB).

4 Conclusions

The physicochemical characteristics of microalgae biomass harvested by the coagulation–flocculation approach were differently impacted by the coagulant used. Relative to non-coagulant harvested DAB, the coagulation process enhanced (*i.e.*, low % transmittance) the intensities of the diagnostic peaks (*i.e.*, peaks synonymous with polysaccharide and protein). The difference in the coagulant for harvesting had no influence on the TP fraction of the DAB. Metal based-coagulants (*i.e.*, $FeCl_3$, $AlCl_3$, and CGS) reduced the concentration of soluble P in the DAB. The concentrations of the soluble-P in the biomass harvested with plant-based coagulants were comparable with the biomass harvested with the CG method. Considering the P speciation in the biomass harvested with metal-based coagulants, they are capable of functioning as slow-release fertilizers. The slow nutrient release potentials of $FeCl_3$-DAB and $AlCl_3$-DAB were attributed to the appreciable amount of Fe/Al–P fraction in them. The presence of Ca_{10}-P was responsible for the slow nutrient release potential of CGS-DAB. Relative to the magnitude of TN present, the metal-based coagulants produced biomass with a higher fraction of available N than the plant-based coagulants. The CG approach produced biomass with the lowest available N. Biomass harvesting using synthetic coagulant had no effect on K speciation.

Conflicts of interest

There are no conflicts to declare.

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