Microalgae Harvesting

Subjects: Plant Sciences

Contributor: Yeek Chia Ho, Teik Hun Ang, Mohammed Bashir

This presents the extractions, characterisations, and applications of natural coagulant in microalgae harvesting. The promising future of microalgae as a next-generation energy source is reviewed and the significant drawbacks of conventional microalgae harvesting. The performances of natural coagulant in microalgae harvesting are studied and outperformed alum. The aim of this work is to elucidate the key aspects for extraction of natural coagulants (plant, microbial and animal) and discussed with justifications. This information could contribute to future exploration of novel natural coagulants by providing description of optimised extraction steps for a number of natural coagulants. Besides, the characterisations of natural coagulants have garnered a great deal of attention, and the strategies to enhance the flocculating activity based on their characteristics are discussed. Several important characterisations have been tabulated in this review such as physical aspects, including surface morphology and surface charges; chemical aspects, including molecular weight, functional group and elemental properties; and thermal stability parameters including thermogravimetry analysis and differential scanning calorimetry.

Keywords: natural coagulant ; microalgae harvesting ; flocculant ; green material ; plant material ; characterisation ; Chlorella vulgaris ; Chlamydomonas sp.

1. Strategy to Enhance Performance of Natural Coagulants in Microalgae Harvesting

After the extraction processes, the final end product is the natural coagulant (plant, animal or microbes). Prior to application in coagulation and flocculation, the characterisation of natural coagulant is vital. Modification of the characteristic of natural coagulant could help in improving its performance in terms of flocculating activity in microalgae harvesting. Table 1 shows the physical, chemical and thermal characteristics of various natural coagulants. Additionally, the performance of various natural coagulants in different application is tabulated in Table 1. Subsequently, the interpretation of these characteristic in related to flocculating activity and their roles in enhancing the performance of natural coagulant in microalgae harvesting are discussed.

Table 1. Characterisation of natural coagulants and its performances.

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	R
Banana peel (*Musa acuminate*)	**-N/A-**	**-N/A-**	**-N/A-**	**C=O, O-H, N–H**	**-N/A-**	**-N/A-**	**-N/A-**	**0.4 g·L^{-1} dosage, 67% removal of chemical oxygen demand (COD) from municipal wastewater**	
Banana pith	**-N/A-**	**-N/A-**	**-N/A-**	**O-H, C-H, C-OO, C-H, COOH**	**O (44%), C (32%), (36 %), H (4.2%), N (1.5%), S (0.86%)**	**-N/A-**	**-N/A-**	**0.1 kg·m^{-3} dosage, pH 4, 99% removal of COD from river water**	
***Brachystegia eurycoma* extract**	**Compact structure with dispersed but continuous crack-like openings, absence of irregular surfaces, randomly formed aggregates and/or loosely bound cluster**	**-N/A-**	**-N/A-**	**O–H, N–H, O=H, C–N, C≡C, C=C–H and H–C–H**	**-N/A-**	**334.44 °C to 361.73 °C**	**−1.708 mV**	**5 g·L^{-1} dosage, pH 8, 97% removal of COD from paint wastewater**	

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	Re
***Brassica spp.* seed protein**	**Pollen grain surface**	**−6.8 mV**	**6.5 kDa**	**-N/A-**	**-N/A-**	**95 °C**	**-N/A-**	**-N/A-**	
Cassava peel starch	**Polygonal and spherical starch granules, rough surface**	**-4.37 mV**	**1.057×10^5 kDa**	**O-H, C-H**	**Ca, K and Na**	**-N/A-**	**-N/A-**	**7.5 mg·L^{-1} dosage, pH 7, 93% removal of total suspended solid (TSS) from dam water 50 mg·L^{-1} dosage, pH 7, 100% removal of *E. coli* from dam water**	
Cactus leaves	**Presence of cracks and cavities**	**-N/A-**	**-N/A-**	**O-H, C=O, COOH**	**Na, K, Ca, Mg**	**-N/A-**	**-N/A-**	**10 mg·L^{-1} dosage, 90% removal of kaolin**	
Cassava Peel (periderm and cortex)	**Non-porous and heterogeneous characteristics, smooth and globular in shape**	**-N/A-**	**-N/A-**	**O-H, CH, CH_2, C=O, C-O, COOH**	**K_2O (5.5%), CaO (4.2%), Fe_2O_3 (1.5%), SO_3 and SiO_2 (0.87%), Al_2O_3 (0.74%), C (0.10%),**	**-N/A-**	**-N/A-**	**-N/A-**	
***Cassia obtusifolia* seed gum**	**Fibrous networks with rough surface and porosity**	**-N/A-**	**-N/A-**	**O-H, C-H, C=O,**	**-N/A-**	**289 °C**	**-N/A-**	**2.47 g·L^{-1} dosage, 82% removal of TSS, settling time of 35.16 min**	
***Ceratonia silique* seed gums**	**Rough cuticle on the adaxial and the abaxial surface, stomatal pores**	**-N/A-**	**5–8 kDa**	**O-H**	**-N/A-**	**-N/A-**	**-N/A-**	**-N/A-**	
Chitin	**Microporous, fish scale shaped nanofibrous surface**	**+18 mV**	**-N/A-**	**N-H, O-H, C-H, C=O**	**-N/A-**	**-N/A-**	**-N/A-**	**0.3 g·L^{-1} dosage, pH 6, 68% removal of turbidity from surface water**	[
Chitosan extracted from lobster shell (*Thenus unimaculatus*)	**Rough surface, irregular block, crystalline with cluster and porosity structure**	**-N/A-**	**-N/A-**	**R-NH_2, O-H**	**Ca, K, Na, Mg and Fe**	**-N/A-**	**-N/A-**	**-N/A-**	
Citrus *Limettioides* peels	**Porous structure**	**-N/A-**	**-N/A-**	**CH, CH_2, CH_3, C=O, COOH, M(RCOO)n,**	**O, Na, Ca**	**-N/A-**	**-N/A-**	**-N/A-**	
***C. obtusifolia* seed gum**	**Rough, fibrous, porous and bulky**	**+6.41 mV**	**-N/A-**	**O-H, C-H, CH_3, CH_2**	**-N/A-**	**280–300 °C**	**-N/A-**	**19×10^{-3} mol gum, 6×10^{-2} mol of NaOH, 87% removal of TSS and 85% removal of COD from palm oil mill effluent (POME) at 50 °C**	

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	R
Cocos nucifera seed protein	Porous structure, clustered, aggregated shapes	-N/A-	5.6 kDa	O-H, N-H	-N/A-	-N/A-	-N/A-	10 $g \cdot L^{-1}$ dosage, 96% removal of As(III) in 8 h, 80 rpm and 50 °C	
Cucumis melo peels	-N/A-	-N/A-	54 kDa	O-H, N-H, CH, CH_2, CH_3, C=O, R–COOH, M(RCOO)n, C-O or –C-N	-N/A-	-N/A-	-N/A-	0.5 $g \cdot L^{-1}$ dosage, pH 7, 91% removal of Mn(II) 0.5 $g \cdot L^{-1}$ dosage, pH 6.5, 91% removal of Pb(II)	[
Cyamopsis tetragonoloba seed gums	Nanoparticles	−6.66 mV	50–800 kDa	O-H	-N/A-	-N/A-	-N/A-	-N/A-	
Dolichos lablab seed gums	Aggregated free, rough	-N/A-	-N/A-	N–H, O-H, C–H, C–C, –COOH	C, O	-N/A-	-N/A-	0.6 $mL \cdot L^{-1}$ dosage, pH 11, 99% removal of turbidity	
Garden cress (*Lepidium Sativum*)	Flake-shaped structures with non-uniform distribution and emerged as interconnected channels, porous and heterogenous characteristics	−16 mV	-N/A-	O–H, C-H, C=O, OCH_3	-N/A-	-N/A-	-N/A-	15 $mg \cdot L^{-1}$ dosage, pH 5, 99% removal of turbidity from river water	
Grafted 2-methacryloyloxyethyl trimethyl ammonium chloride lentil extract	More compact and less porous compared to lentil extract	+15.08 mV	-N/A-	-N/A-	C (62%), O (36%), Cl (2.0%)	-N/A-	-N/A-	5.09 $mL \cdot g^{-1}$ dosage, pH 10, 99% removal of turbidity in surface water and industrial wastewater	
H. esculentus	Compact, cross linkage of molecules	-N/A-	100 kDa	O–H, C–H, C=O	-N/A-	180 °C	36.12 mV	-N/A-	
Kenaf crude extract (KCE)	-N/A-	−8.3 mV	-N/A-	-N/A-	-N/A-	-N/A-	-N/A-	100 $mg \cdot L^{-1}$ dosage, 85% removal of kaolin, 40 $mg \cdot L^{-1}$, 83% removal of turbidity from river water	
Klebsiella pneumoniae	-N/A-	-N/A-	-N/A-	COO^-, O-H, N-H	C, N, O	-N/A-	-N/A-	pH 7, 40% removal of Cd	
Lens culinaris	Rough surface with pores and obvious surface abrasions	−3.58 mV	-N/A-	O–H, C-H, COOH, C=O, C-O	C (60%), O (40%), K (0.39%)	-N/A-	-N/A-	26.3 $mg \cdot L^{-1}$ dosage, 99% removal of kaolin, 3 min settling time	
Lentil extract	Highly porous surface, scattered pieces of compounds attached	−5.91 mV	-N/A-	O–H, C-H, C=O, N-H, C-O-C	C (59%), O (39%)	280 °C	-N/A-	-N/A-	

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	R
Maerua decumbent	-N/A-	-N/A-	-N/A-	O-H, C-H, N-H, C=O, C-O, C-N	C (39%), O (42%) H (3.8%), N (1.2%), S (0.31%)	-N/A-	-N/A-	1 $kg \cdot m^{-3}$ dosage, pH 5.56, settling time 52.31 min, 99% removal of turbidity from paint industry wastewater 0.8 $kg \cdot m^{-3}$ dosage, pH 5.11, settling time 53.53 min, 79% removal of COD from paint industry wastewater	
Malva nut gum	A branch-like surface structure	−58.7 mV	2.3×10^5 kDa	-N/A-	-N/A-	-N/A-	-N/A-	0.06 $mg \cdot L^{-1}$ dosage, pH 3.01, 97% removal of kaolin	
Mango peels	Well-pronounced heterogeneous cavities that are well distributed	-N/A-	-N/A-	O-H, N-H, CH, CH_2, CH_3, C=O, C-O or –C-N	C, H, N, S	-N/A-	-N/A-	-N/A-	
Moringa oleifera	Group-like, composed of many small particles	+6 mV	6.5 kDa	O-H, C-H, C=O, N-H, C-OH, S=O	-N/A-	-N/A-	-N/A-	50 $mg \cdot L^{-1}$ dosage, 94% removal of kaolin	[
Nirmali seeds	highly porous with reticulated structure	-N/A-	12 kDa	COOH, O-H	-N/A-	-N/A-	-N/A-	1.5 $mg \cdot L^{-1}$ dosage, 96% removal of turbidity from surface water	
okra	Porous and rough	−8.3 mV	-N/A-	-N/A-	Mg (7.2%), Al (4.1%), Si (3.7%), P (11.8%), S (8.2%), Cl (7.7%), K (22.0%), Ca (7.5%), O (27.8%)	-N/A-	-N/A-	3 $g \cdot L^{-1}$ dosage, 85% removal of fluoride from hydrofluoric acid synthetic wastewater 20 $mg \cdot L^{-1}$ dosage, 94% removal of kaolin, 40 $mg \cdot L^{-1}$ dosage, 98% removal of turbidity from river water	
Prosopis spp. seed gums	Homogenous in size and shape with a flake-like morphology	-N/A-	62 kDa	-N/A-	-N/A-	Ca, Mg, Fe, Zn	-N/A-	-N/A-	[
Sabdariffa crude extract (SCE)	-N/A-	−6.4 mV	-N/A-	-N/A-	-N/A-	-N/A-	-N/A-	60 $mg \cdot L^{-1}$ dosage, 88% removal of kaolin, 40 $mg \cdot L^{-1}$ dosage, 96% removal of turbidity from river water	
Sago	Smooth and solid surface with no pores	-N/A-	-N/A-	N-H, O-H, C=O	-N/A-	-N/A-	-N/A-	0.1 $g \cdot L^{-1}$ dosage, pH 7, 69% removal of turbidity from surface water	

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	Re
Tannin	-N/A-	−13.6 mV	1250 kDa	O-H, R-NH^2, C=O, COOH	-N/A-	200 °C	-N/A-	14 mg·L^{-1} dosage, 75% removal from kaolin 11 mg·L^{-1} dosage, pH 5 to 7, 97% removal of *Chlorella vulgaris*	
Tamarindus indica seed gums	No fissures, cracks or interruptions	-N/A-	700–880 kDa	-N/A-	-N/A-	97.67 °C	128.40 J/g	15 ppm dosage, 94% removal of turbidity from river water	[
Telfairia occidentalis seed	Coarse fibrous substance largely composed of cellulose and lignin, presence of pores (micro-, macro- and mesopores, compact net structure	-N/A-	-N/A-	O-H, N-H, C=H	-N/A-	-N/A-	-N/A-	247.40 mg·L^{-1} dosage, pH 2, 99% removal of dye in 34.32 mg·L^{-1} concentration with 540 settling time	
T. foenum graecum seed gums	-N/A-	-N/A-	32.3 kDa	O-H, C-H, C=O, N-H, C-OH, C-O-C	C,O	295 °C to 430 °C	-N/A-	-N/A-	
Vegetable tannin	-N/A-	-N/A-	-N/A-	-N/A-	-N/A-	430 °C	-N/A-	pH 7, removal of color and turbidity from dairy wastewater	
Vigna unguiculata seed proteins	Fairly uniform, hexagonal structure, spiked or rugged surface, rough surface, coarse fibrous	-N/A-	6 kDa	O-H, N-H, C=O, C=C-H, C=CH, C-H	-N/A-	-N/A-	-N/A-	256.09 mg·L^{-1} dosage, pH 2, 99% removal of dye of 16.7 mg·L^{-1} with 540 min settling time	[

1.1. Physical Characteristics

The most important physical aspects of natural coagulant that could be studied are surface morphology and surface charges. Surface morphology refers to the imaging of an exposed surface of any object under the microscope, which cannot be seen by the naked eye. By analysing the surface morphology, the active groups attributed to flocculation function can be identified, for example, the citral. According to the Essential Oil-Bearing Grasses, the genus Cymbopogon by Akhila, the oil in citral would help in the blood coagulation–fibrinolysis system [47]. Besides, citral is an antimicrobial element that will protect coagulant such as chitosan from microbial damage [48]. Moreover, the presence of pores (micro-, macro- and mesopores) on natural coagulant could be clearly identified via surface morphology analysis, and they are favourable for the attachment of suspended particles through adsorption, intraparticle bridging or electrostatic contacts during coagulation and flocculation. In addition, the previous study by Obiora-Okafo and Onukwuli [43] proved that a compact net structure coagulant showed higher flocculating activity as compared with a branched structure. Furthermore, changes to the surface morphology of coagulants after coagulation and flocculation show proof of interaction between the coagulants and suspended particles. In view of surface morphology as a strategy to enhance the flocculating activity, modification on physical structures such as grafting could be done to create a high density of pores and ultimately more favourable to coagulation. With these, the mass harvesting of microalgae in the industrial scale is applicable.

On the other hand, surface charge, or zeta potential, is one of the factors that will affect the flocculating activity. Theoretically, zeta potential is the measure of the electrical charge of particles that are suspended in liquid [49]. Practically, the higher the negative surface charge of natural coagulant, the greater it’s flocculating activity against positive suspended particles and vice versa for the positive surface charge of natural coagulant against negatively suspended particles. Thus, the study of surface charge shows a preliminary estimation of flocculating activity of natural coagulant. Besides, the nature of surface charge (positive or negative) indicates the potential treated group of suspended particles, to illustrate, a negatively charged coagulant is used to remove cation heavy metals or the other way round. Chemically and structurally modified of natural coagulant such as quaternary agent 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) grafted on cellulose nanocrystals (CNC) could be applied to enhance the zeta potential to extreme positive or negative [50]. Above all, natural coagulant with positive zeta potential is favourable in microalgae harvesting due to the anionic nature of microalgae.

Moreover, different molecules of the same compound could have different molecular masses because they contain different isotopes with different mass number. The physical aspect of coagulants, molecular weight, could reflect their flocculating mechanism and activity. Yin [8] noted that high molecular weight of natural coagulant played a role in improving aggregation. The higher the molecular weight of natural coagulant, the stronger the bridge formed onto the particle surface than natural coagulant with a lower molecular weight. Thus, the formed flocs were stronger, larger and denser for a larger molecular weight natural coagulant and permitted better settling, also improving the harvesting efficiency [51]. Additionally, the high molecular weight allows natural coagulant's chains to stretch sufficiently far from the particle surfaces; thus, favourable for bridging to form [15]. Another study by Muylaert et al. [52] also showed that the high molecular weight polyelectrolytes (i.e., lignosulfonate) were a better bridging agent. On the other hand, the molecular mass of natural coagulant often reveals its undergoing mechanism in flocculation, for example, the lower molecular weight of natural coagulants, such as polyethyleneamine are usually undergoing flocculation via the charge patch mechanism [52]. It had also been well reported that the high molecular weight of natural coagulant would usually predominant in bridging mechanisms. Yin [8] also suggested that the dimeric cationic proteins with the molecular mass of 12–14 kDa and isoelectric point (pI) between 10 and 11 were predominant in adsorption and charge neutralisation mechanisms. Therefore, by studying the molecular weights of natural coagulants in advance prior to the application, the underlying coagulation mechanism of natural coagulant could be defined and modification could be made based on their respective mechanism. All in all, by knowing the molecular weight, the same compounds can be operated as dispersants (e.g., dextrin, low molecular weight) or coagulants (e.g., starch, high molecular weight). Generally, a dispersant is used to prevent fine particles from aggregating and normally being utilised in a selective flocculation process, in which gangue minerals are dispersed while flocculating valuable or desired minerals [53]. Such approach is suitable to be used in microalgae harvesting.

1.2. Chemical Characteristics

The flocculating activity of natural coagulants also depends on the specific chemical properties of the polymer. One of the key polymer characteristics includes various functional groups. The particular functional groups to be evaluated are COO^- and OH^- as their existence usually contributes to the flocculating activity of natural coagulant. Besides, the increase in positively charged functional groups allows more interactions with the negatively charged suspended particles, and thus improve the binding capabilities of natural coagulants [52]. Modification on functional groups of natural coagulants is also proposed and evinced by researchers in the past studies to increase the flocculating activity. For example, functionalising of cationic starch and TANFLOC, in which, the starch and tannins added with quaternary ammonium groups to increase the flocculating activity and serve as the low-cost as well as more effective alternatives for flocculation process Selective Flocculation Enhanced Magnetic Separation of Ultrafine Disseminated Magnetite OresSelective Flocculation Enhanced Magnetic Separation of Ultrafine Disseminated Magnetite Ores [52]. Additionally, natural coagulants often perform poorly in harvesting marine microalgae [54]. The underlying reason is the high ionic strength of seawater will cause coiling, and this will decrease the effective size of natural coagulants. Therefore, an alternative had been proposed to modify the structure to a more rigid molecule such as tannin-based natural coagulants or functionalised nanoparticles, namely, nanocellulose [52]. Furthermore, in microalgae harvesting, the functional group of natural coagulants can be furthered enhanced with magnetoresponsive Fe_3O_4 nanoparticle to separate the flocculated microalgae from the medium using a magnetic field [55]. To summarise, the modification of functional group begins with characterisation of natural coagulant, which is an important factor that influences the effectiveness of natural coagulant in microalgae harvesting.

Elemental property of natural coagulant affects the flocculating activity. The trivalent cation is the most efficient in flocculating the negatively charged suspended particles. However, trivalent cation is commonly found only in inorganic coagulant such as alum. In plant-based coagulants, divalent cation is predominant instead. Besides, numerous studies have shown that when there are more phenolic groups available in a tannin structure, the coagulation capability could be enhanced [8]. Correspond to this statement, it was reported that the legume-based coagulant was rich in phenolic compounds, and it had also proven to exhibit antibacterial property [28]. These could aid in removing pathogenic bacteria such as *Salmonella parathyphi* that is presented in wastewater due to the leaching of sewage effluents. Thus, phenolic groups provide the –OH group not only for bridging, but to indirectly inactivate the pathogenic bacteria in the wastewater [28]. Therefore, the phenolic group deserves attention in wastewater treatment as well as microalgae harvesting, especially in extraction of DHA microalgae oil. Moreover, there is also one characteristic that has been ubiquitously used as a preselection criterion for new plant-based coagulants, namely, mucilage. Mucilage is a thick, gluey and adhesive substance produced by nearly all plants and some microorganisms. Evidently, the high bridging–coagulation capability of Opuntia with the presence of mucilage will promote the bounding action of particulates to mucilage without directly contact of particulate and has been widely used in water treatment in North America [8][56]. Besides, the recent study on biopolymer coagulant showed that 73% (from 320.0 to 88.0 $mg{\cdot}L^{-1}$) of Fe^{3+} reduction and ~36% of COD removal with an addition of 3.20 $mg{\cdot}L^{-1}$ of okra mucilage during the harvesting process [56]. The presence of galacturonic acid in mucilage will act as an active coagulating agent and provide a bridge for particles adsorption. Further, the partial deprotonation of carboxylic functional group of mucilage in aqueous solution has given rise to the chemisorption between charged particle with COO^- and OH^- [8]. Therefore, it will aid in flocculating activity. To conclude, the selection of natural coagulant for microalgae harvesting should be focused on mucilage as its primary concern.

1.3. Thermal Characteristics

The thermal stability of natural coagulants is also a crucial parameter to be studied in enhancing the flocculating activity. Indeed, an optimum temperature will increase the flocculating activity. However, the temperature higher than 80 °C will usually destroy the chemical composition of natural coagulants [57]. Moreover, the temperature has direct effects on floc formation, breakage and reformation. To illustrate, floc formation is slower at a lower temperature, whereas breakage of floc is greater at higher temperatures. On the other hand, thermogravimetry analysis determines the minimum temperature causing decomposition of organic components in natural coagulant and differential scanning calorimetry allows study relating to the heat flow required to decompose the natural coagulant. In general, the thermal characteristics reveal the thermal stability of natural coagulant and it has no direct impact on microalgae harvesting because coagulation will not occur in extreme temperature.

2. Application of Natural Coagulant in Microalgae Harvesting

In the previous section, the extraction and characteristic of natural coagulant, as well as the strategies to enhance its flocculating activity, are reviewed. In this section, the application of natural coagulant in microalgae harvesting will be the focal point. To recall, alum always appears to be the first option in industrial applications when comes to the selection of coagulant for microalgae harvesting. The reason being, it is widely available, it promotes coagulation by neutralisation and most importantly it is ready to be dissolved with water.

However, the emerging usage of plant-based coagulant has achieved higher harvesting efficiency compared with chemical coagulant and there are reviews on their effectiveness and relevant coagulating mechanisms for the treatment of wastewater and microalgae harvesting [56][58][59]. To illustrate, the plant-based coagulant could be applied on microalgae harvesting at relatively low cost [60]. Compared to alum, the natural coagulant is deemed to be environmentally friendly because it is extracted from plants, animal or microbial and usually existed in non-toxic form [61]. The water soluble active compound in natural coagulant will be removed after several cycle of kidney filtration, leaving less possibility of producing toxicity in the body [62]. In view of sludge production after the harvesting process, natural coagulant does not produce suspended alum residual and indeed produces less organic residual due to its biodegradability. In contrast, alum requires chemical reaction to break down and will not decompose naturally. In a specific type of microalgae harvesting, for instance, extraction of DHA rich microalgae oil as a dietary supplement, natural coagulant appears to be the best option as it harvests a higher amount of microalgae biomass compared to alum and at the same time, it is safe for consumption. Thus, it will not pose any health concern even there is residual remained in algae biomass. The natural coagulant is proven to achieve higher flocculating activity in comparison to alum and their performance is shown in Table 1 and Table 2. In addition, by utilising the natural coagulants, it reduces the alum dependency and ultimately achieves sustainability in the microalgae-based biofuel production industry as well as various fields, including wastewater treatment and medical to name a few. Figure 1 shows the advantages of natural coagulant in microalgae harvesting.

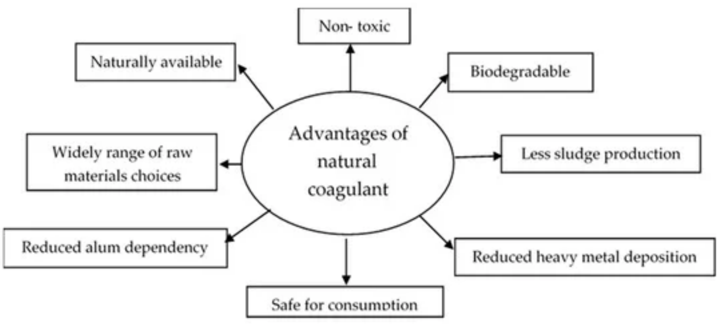

Figure 1. Advantages of utilising natural coagulant in microalgae harvesting.

Furthermore, natural coagulants have also been proven by other researchers as an effective way to harvest microalgae. It was found that the usage of bio-coagulants for harvesting microalgae could eliminate the toxicity contamination on harvested microalgae biomass [63]. The study carried out by Tran et al. [64] to harvest *Chlorella vulgaris* with alkyl-grafted chiton Fe_3O_4–SiO_2 showed 90% of biomass removal by merely employing 0.013g·L^{-1} dosage. On the other hand, a plant-based coagulant, *M. oleifera*, showed a 76% of harvesting efficiency on *Chlorella* sp. biomass after 100 min with 8 mg·L^{-1} dosage and 96% of harvesting efficiency in 20 min when combining *M. oleifera* with chitosan [65]. Furthermore, 60% of microalgae removal efficiency was achieved with 12 mg·mL^{-1} of *F. indica* extract after 120 min of settling time [66]. To sum up, the utilisation of natural coagulants in microalgae harvesting is a trend of research in the past few years. Unfortunately, it was set up and investigated merely at a laboratory scale. Table 2 shows the application of natural coagulants on microalgae harvesting.

Table 2. Application of microalgae harvesting using natural coagulants.

Natural Coagulant	Operating Condition	Performance	Reference
Alkyl-grafted chiton Fe_3O_4–SiO_2	**0.013 g·L^{-1} dosage**	**90% removal of *Chlorella vulgaris***	[64]
M. oleifera	**8 mg·L^{-1} dosage**	**76% removal of *Chlorella vulgaris***	[65]
***M.oleifera* with chitosan**	**8 mg·L^{-1} dosage**	**96% removal of *Chlorella vulgaris***	[65]
F. indica	**12 mg·mL^{-1} dosage**	**60% removal of microalgae**	[66]
Pleurotus ostreatus strain HEI-8	**pH 3, glucose content 20 g·L^{-1}, fungi pelletisation time 7 days, 100 rpm**	**65% removal of *Chlorella* sp.**	[67]
Citrobacter freundii* (No. W4) and *Mucor circinelloides	**pH 7, glucose concentration 1.47g·L^{-1}**	**97% removal of *Chlorella pyrenoidosa***	[68]
Tannin	**11 mg·L^{-1} dosage, pH 5 to 7**	**97% removal of *Chlorella vulgaris***	[69]
Tannin	**5 mg·L^{-1} dosage, pH 7**	**80% removal of *Oocystis* microalgae**	[70]
Eucalyptus globulus	**20 mg·L^{-1} dosage**	**95% removal of Scenedesmus sp.**	[71]
***Cassia* gum**	**80 mg·L^{-1} dosage**	**93% removal of *Chlamydomonas* sp.**	[72]
***Cassia* gum**	**35 mg·L^{-1} dosage**	**92% removal of *Chlorella* sp.**	[72]

As an additional point, statistical modelling approaches could be studied to identify the optimum operating condition of natural coagulant. After several trials in the coagulation process, a statistical approach such as linear regression method is feasible in extracting the optimum parameters of natural coagulant in coagulation with collected data and equations.

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