

Review

Insight on Extraction and Characterisation of Biopolymers as the Green Coagulants for Microalgae Harvesting

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Abstract: This review presents the extractions, characterisations, applications and economic analyses of natural coagulant in separating pollutants and microalgae from water medium, known as microalgae harvesting. The promising future of microalgae as a next-generation energy source is reviewed and the significant drawbacks of conventional microalgae harvesting using alum are evaluated. The performances of natural coagulant in microalgae harvesting are studied and proven to exceed the alum. In addition, the details of each processing stage in the extraction of natural coagulant (plant, microbial and animal) are comprehensively 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. Furthermore, various applications of natural coagulant in the industries other than microalgae harvesting are revealed. The cost analysis of natural coagulant application in mass harvesting of microalgae is allowed to evaluate its feasibility towards commercialisation in the industrial. Last, the potentially new natural coagulants, which are yet to be exploited and applied, are listed as the additional information for future study.

Keywords: natural coagulant; production; characterisation; application; microalgae harvesting; cost analysis; coagulation and flocculation

1. Introduction

The fast-developing countries rely heavily on large-scale industrialisation to improve their global economic competitiveness. Concurrently, the growing amount of waste produced in modern society has become a global issue. In many contexts, developing countries have produced tons of wastes from industrial revolution. As the world is moving towards green technology, natural coagulant, which can be extracted from plant tissues, animals or microorganisms, has been a major point of interest. Notably, studies from past researchers have shown the effectiveness of natural coagulant in wastewater treatment, such as turbidity removal through neutralisation of anionic suspended particles with cationic polymers. To provide a more focused discussion, coagulation is an important process in surface water treatment, for example, *Moringa oleifera* has been used in native communities in treating river water for drinking purpose. On the other hand, natural coagulant could take place in treating commercial wastewater, for instance, *Maerua decumbent* has been used to treat paint wastewater, which marked 99% of turbidity removal by using $1 \text{ kg}\cdot\text{m}^{-3}$ in dosage [1].

Recently, natural coagulant has also emerged as a promising solution in microalgae harvesting as it will not create by-product, such as suspended alum residual in microalgae biomass, which is needed to be further removed before lipid extraction. Concurrently, natural coagulant requires a lower dosage in mass harvesting of microalgae compared to alum. Natural coagulants are usually used as point-of-use products in less developed countries because they are fairly cost-effective as compared with the alum and could be easily processed in the usable form [2]. Moreover, natural coagulant gains advantages over alum in terms of reduced sludge production, produces treated water with less extreme pH and it is in line with sustainable development. The use of natural plant-derived materials to coagulate and flocculate microalgae biomass produced in mass cultivation system are not a new idea, for instance, natural coagulants have been used to clear turbid water since ancient times, even before the emergence of chemical coagulants [3]. In view of microalgae cultivation, the most common approach is the suspended growing method as it allows the microalgae to distribute evenly in the medium for nutrient intake. In contrast, a non-suspended mode of cultivation allows microalgae to grow on the surface to form a biofilm. It is more commercially feasible because the harvesting process of microalgae biomass will be easier. The typical example of non-suspended microalgae are *Scenedesmus obliquus* sp. and *Botryococcus braunii* sp. [4].

Apparently, natural coagulant is an emerging solution to green and sustainable water treatment. Besides microalgae harvesting, it has been utilised on various new sectors, for example, electro-coagulation in microbial fuel cell system to precipitate heavy metals for self-power system [5] and membrane manufacturing plant wastewater treatment [6]. Moreover, natural coagulant has also been proven to remove 97% of copper ions in 3 h from wastewater of rotating triboelectric Nano generator (R-TENG), to remove lead (II) ion by 88% at pH 5 from simulated wastewater using cashew nut coagulant [7], to remove wastewater by coupling coagulation using anion exchange membranes (AEMs) in electrodialysis [8] and last to be used in bioreactor for aerobic sewage wastewater treatment using natural coagulant (micro-based coagulant) such as *Bacillus* species, *Achromobacter* species and *Comamonas* species. [9].

There is also strong evidence that the use of biopolymer and plant-based materials has been increasing and penetrating into various fields, for example, the technology of reusing cysteine-containing protein materials from keratinous waste to produce tough keratin fibre [10], fabrication of sustainable membrane using bamboo fibre to enhance cross-flow filtration performance [11] and perforated lotus leaf to treat oil spillage [12]. Besides, biopolymer is also widespread in other fields such as utilisation of natural fatty acids for drug releases in the medical field [13], biophenol coatings on nanofiltration membranes to improve its performance on the separation of organic media [14] and glucose-based biopolymer to modify the interlayer of the solar cell, which enables 95% of enhancement in power conversion efficiency [15].

Prior to the application, the extraction of natural coagulant from plant, animal or microbes are needed. However, the current extraction method poses a significant drawback, which is time

consuming as it involves several stages of pretreatment. The preparation stages associated with each type of natural coagulant (plant, microbial and animal) are varied as well. In addition, each plant, animal or microbial coagulant has different optimum extraction methods. Sometimes, water extraction of natural coagulants commonly used by native communities could be further incorporated with currently employed techniques such as salt and acid extractions to maximise the extraction efficiency.

Furthermore, the previous papers are mainly focused on the performance of natural coagulant in coagulation and flocculation, for instance, the optimum operating condition of 21 types of plant based coagulants and their barrier to commercialisation [16]; the optimum operating conditions of *Dolichas lablab*, *Azadirachta indica*, *Moringa oleifera*, *Hibiscus rosa sinensis* [17]; and the modification of functional group of natural coagulants in enhancing the flocculating activity [18]. Thus, to provide a more comprehensive discussion, this review will include the technical aspects, such as the extraction processes of natural coagulant with detailed explanations on its necessity, because the extraction stage is as important as the performance stage and should not be neglected. By reviewing the extraction processes step by step, further studies on discovering the new natural coagulant will be easier because the relevant extraction processes could be referred here based on their nature of characteristic. To illustrate, the study of extraction method of new coagulant, Aloe Vera gel, could be referred to Aloe Vera as they are from the same genus. Besides, the recent trend of research is mainly on plant-based coagulant; thus, in this review, further explorations on animal- and microbe-based coagulants are carried out and their optimum operating conditions are technically discussed. Characterisation of natural coagulant is explored as well in accordance with Ang [18], covering additional information such as surface morphology, molecular weight, zeta potential, TGA and others. The promising future of microalgae as next generation of energy is presented with the application of natural coagulant in microalgae harvesting, followed by its advantages and disadvantages with respect to alum. In summary, natural coagulants derived from plants, microorganism and animals are reviewed for their extractions, characterisations and applications in microalgae harvesting. Cost analysis of natural coagulant for large scale application in industrial is carried out to provide an appropriate platform for future researchers to intensify on microalgae harvesting by using these natural materials.

2. The Promising Future of Microalgae

2.1. Microalgae as Next Generation of Biofuel

Renewable energy plays a vital role in energy resources, and access to green and cheap energy has become a trend in modern society. Biofuel is a type of renewable energy in a form of liquid and gaseous fuels produced from biomass, namely bio-ethanol, bio-methanol, bio-oil, bio-diesel and bio-gas. Generally, there are four types of biofuel generation and their characterisations are based on the nature of the feedstock. The first generation of biofuel is extracted from food crops, for example, from sugarcane through chemical process such as fermentation. However, a series of problems regarding to fuel vs. food dilemma have been attributed to the production of the second-generation of biofuel, in which its extraction is from non-food crops. Likewise, the second generation of biofuel does experience unexpected demise as the first generation of biofuel. Arnold et al. [19] noted the innovation of second-generation biofuel was relatively constant in the mid-1990s and followed by decline in the following years. The long-term development of the second generation of biofuels is a step in the right direction; however, it has several drawbacks such as cost effectiveness and technological barriers in dealing with biomass [20,21]. To address these concerns, the third generation of biofuel, which is derived from microbes, has been introduced.

Microalgae is touted to be a sustainable energy source of the third generation of biofuel. The Solar Energy Research Institute, USA, has proposed microalgae as an intermediate tool for biofuel production since the 1940s [22]. In comparison with other energy crops, microalgae biofuel has the advantage of quick growth, high lipid, carbohydrate content and excellent biomass yield with the lowest capacity of land used [23]. Previous studies have established that microalgae can be the replacement of fossil

fuel due to its high amount of intracellular accumulated oils [20]. Additionally, the cultivation of microalgae using waste is the essence of the research vanguard. In view of sustainability, cultivation of microalgae using waste such as Palm Oil Mill Effluent (POME) is an added point to the environment.

Besides, the strong requirement for clean energy production and conversion technology development at a global scale led to mass researches on microalgae as a feedstock to generate biofuel. Developed nations, for instance, the USA, Australia and Mexico have focused their researches towards the efficient cultivation of microalgae and simultaneous wastewater treatment in the past few years [24]. To further enhance biofuel production, the fourth generation of biofuel, which uses genetically modified microalgae in production, has attracted enormous attention. The improvement in metabolic activity, photosynthesis efficiency, light penetration and reduction of photo-inhibition of genetically modified microalgae lead to enhancement of fourth generation of biofuels in term of quality and quantity [25].

Further, comparative studies evinced that microalgae also help in absorbing carbon from the industrial gases and utilisation of nitrogen and phosphorus from industrial and municipal wastewater [23,26]. At present, microalgae are competitive and are becoming a trend of future energy resources.

2.2. Bioprocess Approach of Microalgae Biofuel

The bioprocess approaches of microalgae biofuel are divided into four phases: (1) microalgae cultivation, (2) harvesting, (3) cell disruption and extraction and (4) fatty acid profiling [27]. In the cultivation stage, the selection of a cultivation medium is relatively important. Cultivation medium is a source of energy, nutrients or growth factors that design to grow certain targeted species. The favourable medium for microalgae growth consists of nutrients such as nitrogen and phosphorus, moderate pH, feasible to light penetration and allow CO₂ circulation. Therefore, several wastewaters, such as POME, rubber mill effluent and landfill leachate, have been studied to be used as cultivation medium, with the condition that nitrogen and phosphorus are present in the composition [20,23,24]. Alternatively, there are many standard solutions available in the market, prepared for the cultivation of microalgae, called standard cultivation medium. Bold's Basal Medium (BBM) is one of the mediums consisting of (1) 10 mL per litre of culture medium with the following chemicals, sodium nitrate (25 g·L⁻¹), calcium chloride dihydrate (2.5 g·L⁻¹), magnesium sulfate heptahydrate (7.5 g·L⁻¹), dipotassium phosphate (7.5 g·L⁻¹), monopotassium phosphate (17.5 g·L⁻¹) and sodium chloride (2.5 g·L⁻¹) [20]. Noteworthy, the starvation phase in the pre-harvesting cultural stage is also proven to trigger the accumulation of lipids after the stage where microalgae growth is maximised [28].

Subsequently, the harvesting of microalgae is the main focus of this review. Indeed, alum is mainly used as a coagulant in microalgae harvesting industrial and the usage of natural coagulant is limited to academic research. However, an obvious drawback has arisen in conventional microalgae harvesting as the alum will result in extreme pH of the treated end product, especially in mass harvesting of microalgae biomass with the addition of a huge amount of alum. Some of the reflections are gathered and stated that using alum with coagulants is ineffective in low temperature, and has high procurement costs and detrimental effects on human health [29]. It has, somewhat, been noted that the mass harvesting process faces drawbacks such as the reduction in lipid due to the addition of alum [30]. Further, alum might be the cause of Alzheimer's disease, which deposition of alum in body has significantly impact to our health [2]. In some aspects, such as the harvesting of EPA/DHA enriched microalgae oil using alum might result in a high aluminium level in microalgae oil. Thus, an alternative solution is sought, and natural coagulants are deemed and anticipated to overcome this problem. Subsequently, optimum microalgae recovery could be carried out for mass production.

Compared to alum, the usage of natural coagulant in the harvesting process is promising due to its non-toxic nature and that it is safe for consumption [31]. Therefore, progressive research on natural coagulant in microalgae harvesting should be done, especially for EPA/DHA dietary microalgae oil. To date, the disadvantages of natural coagulant are mainly due to its feasibility in terms of production

time, commercialisation in industrial and quality control. Figure 1 shows the disadvantages of utilising natural coagulant in microalgae harvesting.

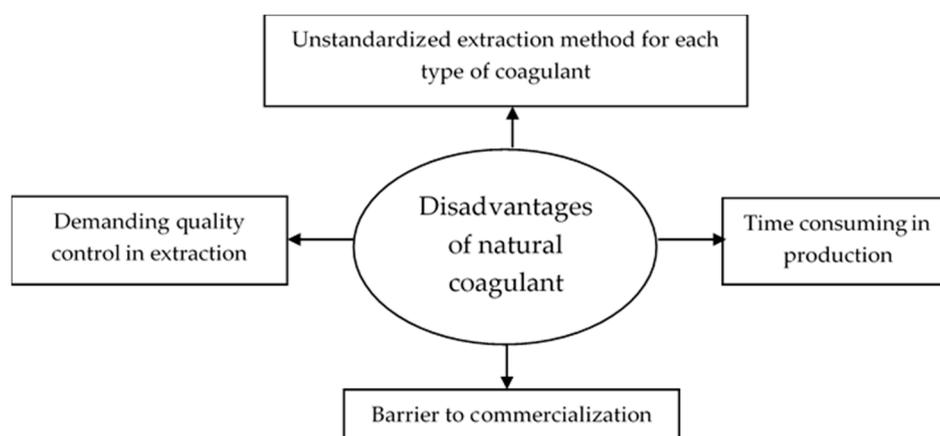


Figure 1. Disadvantages of utilising natural coagulant in microalgae harvesting.

In sum, the extraction process of natural coagulant from plant, animal and microbes is complicated and time-consuming. Different optimum extraction methods for each type of natural coagulant further disrupt the commercialisation of natural coagulant in the industrial.

Up to now, studies have shown progressive optimisation in the extraction process of natural coagulant. Significantly, the extraction process could be enhanced and modified based on domain knowledge. In this review, justifications on the necessity of each sub-steps to be carried out in the extraction process of natural coagulant (plant, animal and microbial) are discussed. Notably, this information will help in understanding of the concept of extraction and also provide references for the extraction of new natural coagulant in the future.

Furthermore, it is also technically proven that flocculating activity could be increased by modifying the characteristic of natural coagulant. Thus, this could be applied to all types of natural coagulant to offset the disadvantages as mentioned. To address this, strategies to enhance the flocculating activity of natural coagulant based on their physical, chemical and thermal characteristic have been espoused in Section 4.

3. Extraction of Natural Coagulants

3.1. Plant Based Polymers

Over the past few years, researches have been conducted on various types of natural coagulants derived from plant wastes and fruit pieces, for instance, Nirmali seeds, *Moringa oleifera*, Surjana seed, Arabic gum, maize seed, tannin, Cactaceae, etc. had demonstrated significant coagulant capacities [32,33]. Among all, the plant-based coagulant recently received the greatest level of attention is the seed of *Moringa oleifera* native to Sudan [2]. Research by Vijayaraghawan et al. [34] shows that the water extracted *M. oleifera* seed has a comparative result with aluminium salt (alum). Moreover, there are standardised and well organised extraction steps in extracting the plant-based coagulant [35]. The general processing steps of the extraction of plant-based coagulants can be categorised into three major stages: primary, secondary and tertiary (Figure 2). Further, there is green extraction technology, for instance, utilisation of salt solvent will increase the extraction efficiency and flocculating activity of peanut seed coagulant as compared to water extraction, which has an improvement of 61% in turbidity removal [36]. Ultimately, it will reduce the cost of extraction and energy used along the harvesting process.

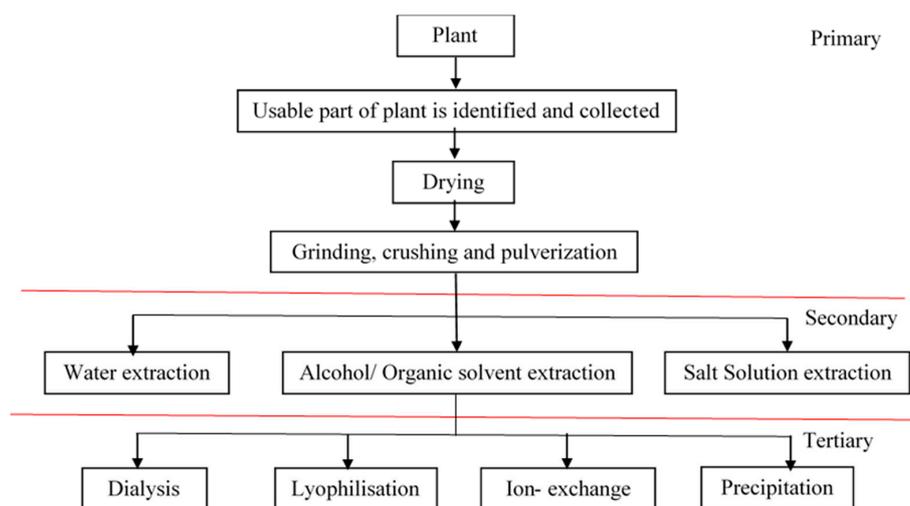


Figure 2. General processing steps in plant based coagulant extraction.

3.1.1. Primary Processing

The primary processing stage involves choosing of usable parts of the plant. The important factor to be considered in the choice of usable part is associated with their respective coagulating properties. In the case of cacti, the usable part is the vascular tissue of the plant, and therefore the skin and spines are eliminated. However, the usable part of aloe species is different, which are the mature leaves and the perimeter spines. At this stage, there must be no sign of contamination, parasites, presence of external insects and organisms on the surface of plants [35]. Furthermore, the selected usable part must be washed with plenty of water to eliminate impurities such as sand stones or grain wastes and to prevent the presence of fungi and yeasts due to bulk handling, fractionation and packaging [35]. In regard to this, the authors of [37] have introduced formaldehyde or known as acid–alkaline wash of plant, which significantly enhanced the pretreatment phase of natural coagulant extraction. The acid helps in removing the minerals on the surface while the alkali acts as neutralising agent to acid. These solvents had been proven to remove organic materials and in the same way, it is traditionally used in the ion-exchanged technology. Therefore, by utilising acid–alkaline wash, it can be assumed that certain degree of organic materials on the surface of plant had been removed prior to the secondary processing and this ultimately reduces the leaching of organic matters inside the usable part. The presence of organic matter will have negative impact on drinking water treatment such as causing colour, odour and taste problem. In the drying stage, the materials are ubiquitously carried out at oven or outdoor to evaporate the water content and reduce moisture level. The presence of water will affect the extraction, while dried plants will reduce the possibility of further enzymatic or metabolic alteration of plant. It is important to be carried out in warm tropical climate with temperature ranges between 20 °C and 35 °C, low humidity between 50% and 70% on average and most importantly, it is highly advised not to dampen by rain or other water sources [35]. Afterwards, the crushing, mechanical grinding and powdering of the dried extract could be carried out with machine followed by passing through a mill to pulverise the material [35]. Ultimately, it is sieved to obtain a very fine powder and stored in airtight containers to avoid hydration prior to the subsequent use in secondary processing of coagulants [35].

3.1.2. Secondary Processing

In the secondary processing stage, the active coagulating agents of each plant could be extracted via different solvents (organic, water or salt solutions). This comes as a surprise at first glance as each type of plant has a unique chemical structure and electrostatic properties providing novelty. Additionally, different solvents could be used in sequence at the secondary processing stage, for example, solvent extraction of valuable and edible oil from *M. oleifera* (MO) seed [38] followed by water extraction of active component for coagulants from *M. oleifera* (MO) seed waste. In the African countries, MO seed

residue as a by-product of oil extraction is used to extract natural coagulant for water treatment [2]. Indeed, the oil content in MO seed is not attributed to flocculating activity and the oil content will actually affect the performance of natural coagulant especially in heavy metal removal activities [39]. The oil will reduce the efficiency of coagulation by making re-stabilisation of destabilised particles and ultimately reduce the binding sites for coagulation. Therefore, the oil content in each plant should be processed through a pre-secondary treatment [39]. In many cases, the extraction using water is evidently the most accepted option due to its abundance and cost-effectiveness provided that the plant's active component is water-soluble protein [33].

The application of salt solution extraction is rather recent and more effective compared to the water extraction method. It is found that the coagulation capacity of the MO using salt solution extraction is 7.4 times higher than the water-based extraction in the study of the removal of suspended kaolinite [40]. To illustrate, the delipidation is involved in the salt extraction process and this will lead to least possible of lipid content in the extracted MO active component. Ultimately, the decrease in lipid will result in the increase in coagulation capacity [40]. The previous study by Ndabigengesere, Narasiah and Talbot [34] was first proposed that one of the disadvantages of water-based extraction of MO was the increase in dissolved organic carbon (DOC) residual of the treated water. The DOC is usually due to the presence of organic materials and a precursor of disinfection by-products in drinking water treatment. The presence of DOC could result in an increase in chemical oxygen demand (COD). The increase, however, does not affect the salt solution extraction due to the salting-in mechanism where increasing of ionic strength of a solution will increase the solubility of the solute.

Nonetheless, significant setback emerges because the prepared powder (biopolymer) contains not just the coagulating active agents, but also plant tissues. The latter is rich in plant tissue, thereby increasing the organic loading in the treated water, which may exacerbate the situation further rather than improving the efficiency of treatment after coagulation and flocculation [38]. This problem can be addressed by processing the powder through tertiary (purification) stages.

3.1.3. Tertiary Processing

The tertiary processing is rarely performed and is limited to academic research [34,38] as this increases the overall processing cost. After the secondary processing stage, the active coagulating agents appear as supernatants in the solution. Preliminary studies suggested that dialysis, lyophilisation and ion-exchange were feasible purification methods in tertiary processing stage. A recent review of literature on this topic found that the oldest and simplest coagulant recovery technologies are solid-liquid filtration and settlement to remove just gross solids from the extracted coagulant [41]. All of these are still applied in industrial applications; however, modern technologies do discriminate natural coagulants from contaminants by molecular size and charge. These principles have been applied using membranes and adsorbents. Certainly, there are several studies on tertiary recovery of coagulant using ultrafiltration (UF) at the bench as well as pilot scale [42,43]. In these studies of tertiary recovery, the rationale was to select ultrafiltration pore sizes that allowing trivalent metal to penetrate while retaining natural organic material. Further, membranes with a molecular weight cut-offs of 10 kDa allowed aluminium permeation to exceed 90% and total organic carbon rejections of 50–66% [44]. Although it is not extensively reported in past researches, the main drawback of ultrafiltration was the fouling and quality issues. In other words, the molecular weight, functionality and nature of organic compounds are varied widely and depended heavily on environmental conditions and heavy metals do have similar cationic and molecular weight characteristics as natural coagulants. Due to the overlap in molecular weights of natural coagulants and organic contaminants, researches proposed the coagulant separation technologies using molecular charge as the principal means to differentiate the cationic coagulant from anionic or neutral contaminants. The tertiary recovery process of ionic exchange has been in the form of ion-exchange media such as liquids, resins, and dialysis membranes [41]. Besides, it is recognised, for example, *M. oleifera* is highly biodegradable natural coagulant with a very limited shelf life. Lyophilisation, often known as freeze-drying, is a technique used to retain biological material

by freezing the extraction mixture, extract the supernatant (natural coagulant), then drying at quite low temperatures through a vacuum. The relevant study also showed that the freeze-dried *M. oleifera* retained its high coagulation efficiency for up to 11 months regardless of storage temperatures and packaging methods [45,46].

3.2. Microbial Based Polymers

Apart from plant-based coagulants, there are coagulants produced by bacteria and fungi. Particularly, different microorganisms could yield different flocculating coagulant from their respective bacteria strain, i.e., proteoglycan coagulants (98% polysaccharide and 1.6% protein) is yielded from *Bacillus mojavensis* strain 32A with an interesting flocculating activity of 96% recorded at pH 10 [47]. Various factors have to be considered in the selection of bacteria. The predominant step in the preparation of microbial-based coagulant starts with the preliminary screening of bacterium strain based on its mucoid and rosy colony morphology characteristics. It is then followed by the biochemical identification of the strain based on 29 biochemical and enzymatic reaction tests (BBL Crystal Gram-Positive ID System). After the identification of the microbial-based coagulants from bacteria strain, batch cultures are prepared to cultivate bacteria and produce natural coagulant at room temperature. Subsequently, the flocculating activity of each natural coagulant is determined via kaolin assays [47]. It had been observed that variation in cultivation medium of bacteria would affect the growth of microorganisms and its ability in producing the expected exopolysaccharides or natural coagulant [48]. Researches had been performed to identify the bacterium strain that aid in flocculating activity and shown in Table 1. General preparation processes of microbial-based coagulant are summarised as below.

1. Preliminary identification of the natural coagulants-producing bacterium strain based on its mucoid and rosy colony morphology characteristics.
2. Screening of bacteria and fungi to find microbial-based coagulants from bacterium strain.
3. Determining the flocculating activity of microbial-based coagulants (natural coagulants) yielded from each bacterium strain by kaolin clay suspension.
4. Optimising the culture conditions of bacteria to produce a higher amount of natural coagulant.

Table 1. Microbial strains and their respective flocculating activities.

Bacterial Strain	Flocculating Activity in Removal of Kaolin (%)	Reference
<i>Bacillus agaradhaerens</i> C9	81	[49]
<i>Bacillus</i> sp. XF-56	94	[49]
<i>Arthrobacter</i> sp. B4	99	[50]
<i>Bacillus licheniformis</i> X14	98	[47,51]
<i>Bacillus velezensis</i> 40B	>98	[52]
<i>Chryseobacterium daeguense</i> W6	97	[48]
<i>Klebsiella</i> sp. ZZ-3	95	[53]
<i>Streptomyces</i> sp. MBRC-91	96	[54]
<i>Aspergillus flavus</i> (source NI 3)	>90	[55]
<i>Penicillium</i> strain HHE-P7	96	[47,56]
<i>Aspergillus flavus</i> (source NI)	97	[55]
<i>Rhizopus</i> sp. M9 & <i>Rhizopus</i> sp. M17	90	[57]
<i>Talaromyces</i> sp.	93	[58]

There are several screening methods that could be applied on testing of a bacterium strain. A colorimetric method is an approach to determine the concentration of chemical compounds with the aid of colour. Further, optimisation of cultivation medium of bacteria and fungi could be conducted using statistical analyses, which discovers the pattern and trend of bacteria growth with equation. Experimental design of various microbes is carried out by cultivating them in different sources of nutrients to produce natural coagulants with different characteristics. These data are collected and

useful for interpretation by a statistical linear regression method to find the relationship between each factor and ultimately lead to production of higher amount of natural coagulant.

Besides, the essential nutrients for microbial growth are mainly carbon and nitrogen elements. At the same time, wastewater and sludge are abundant with carbon, nitrogen, phosphorus and micronutrients, which could sustain the microbial growth for natural coagulants production. In this context, studies also showed that agro-industrial wastes, such as sugarcane, starch molasses, corn-steep liquor, soybean juice, etc., which are mainly composed of polysaccharides, could be used for microbial growth for natural coagulants production. To sum up, the optimisation in cultivation medium of each type of bacteria is different and should be studied accordingly through experimental works.

3.3. Animal Based Polymers

The animal-based coagulant is derived mainly from chitin, which is a natural polymer from two marine crustaceans, namely, shrimp and crabs. Chitin, the most common polysaccharide after cellulose, is a non-elastic and nitrogenous natural polymer structured as a linear chain by the 2-acetoamido-2-deoxy- β -D-glucopyranose monomers [59]. Chitosan-based materials are the potentially eco-friendly coagulants and flocculants in harvesting process because of their natural biological characteristics and biodegradability. Generally, the mechanism involved in the harvesting process of chitosan is bridging. Chitosan is commonly used in laboratory for microalgae harvesting, for example, to harvest *Chlorella* sp. from its cultivation medium [60]. Furthermore, its advantages of recyclability and as an excellent chelating agent for arsenic, molybdenum, cadmium, chromium, lead and cobalt ions make it an excellent choice for industrial wastewater treatment [60]. Table 2 shows the flocculation abilities of chitosan at its optimum operating conditions in removing various pollutants or separating microalgae.

Table 2. Flocculation abilities of chitosan at its best conditions to separate various pollutants from the aqueous medium.

Chitosan	Operating Condition	Flocculation Ability	Reference
Chitosan	214 mg·L ⁻¹ , pH 8 and 131 rpm	92% removal of <i>Chlorella vulgaris</i>	[61]
Chitosan (<i>Plaeomon serratus</i>)	15 mg·L ⁻¹ at 67 nephelometric turbidity units (NTU) raw water, flocculation time of 20 min	89% removal of sewage wastewater	[62]
Chitosan (shrimp)	4 mg·L ⁻¹ , pH 6 and flocculation time of 10 min	95% removal of <i>Thalassiosira pseudonana</i> microalgae	[63]
Chitosan	30 mg·L ⁻¹ , pH 7, flocculation time of 20 min	98% removal of <i>Chlorella vulgaris</i>	[64]
Chitosan	4 mg·L ⁻¹ , pH 4 and flocculation time of 10 min	90% removal of <i>Thalassiosira pseudonana</i> microalgae	[63]
Chitosan	20 mg·L ⁻¹ , pH 9.9, flocculation time of 10 min	90% removal of <i>Thalassiosira pseudonana</i> microalgae	[63]
Xanthated chitosan	50 mg·L ⁻¹ , pH 6.0, slow stirring for 10 min and settling for 10 min	>97% removal of Cu ²⁺	[65]

However, chitosan is insoluble in either water or solvent. Thus, diluted acids such as acetic acid and hydrochloric acid are used. When acid is added, the free amino groups are protonated and the biopolymer becomes fully soluble [66]. Most of the preparation techniques of chitosan rely on chemical processes for extracting the protein and removing of inorganic matter. The processes involved extraction by solvent, followed by oxidation of remaining residues [67]. Overall, the extraction of chitosan from raw material includes the following stages; (1) grinding of raw materials (processing), (2) translating the mineral components of raw material into the soluble form (demineralisation), (3) removing the protein fractions (deproteinisation) and (4) deacetylating of chitin in obtaining the chitosan (Figure 3).

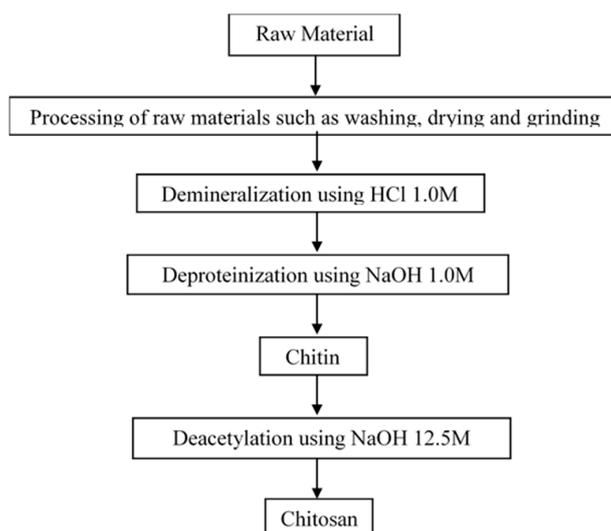


Figure 3. Flow of conventional preparation of chitosan.

The first step in the extraction of chitosan is the processing of raw materials, e.g., crab shell is removed from crab, washed, dried, grinded and filtered before it can proceed to demineralisation. During the demineralisation process, both metal ions and salt anions are removed via ion exchange. In this process, strong acid cation in the form of H^+ converts the dissolved salts into their conjugate acids. Demineralisation involves three sub-steps: (1) reaction of shell powder with hydrochloric acid to release carbon dioxide bubbles, followed by (2) washing using distilled water and (3) oven drying [59]. In addition to the removal of hardness in demineralisation stage, this process removes all dissolved solids such as sodium, silica, alkalinity and the mineral anions. Deproteinisation is carried out right after the demineralisation. By definition, deproteinisation is a process of removing protein and various enzymes in the sample prior to extraction of chitosan. As a cleaning agent, sodium hydroxide saponifies fats and dissolves proteins. Moreover, its hydrolysing power can be further enhanced with the presence of chlorine [68]. The change in colour of sodium hydroxide to clear indicates an index of full deproteinisation [59]. Prior to deacetylation stage, the precipitant must be drained and washed with distilled water repeatedly until its pH is dropped to neutral. Traditionally, deproteinisation and demineralisation steps are repeated twice to aid in higher yield of chitin from the shells. The last step is deacetylation, which refers to the process of removing acetyl groups. In general, alkali could be used to partially deacetylate chitin to produce a mixture of chitin and chitosan. As compared with chitin in terms of chemical structure, chitosan only lacks in acetyl group. Thus, deacetylation is a process of removing acetyl group. Deacetylation started by dissolving the demineralised and deproteinised product (chitin) in high concentration of sodium hydroxide. Heating can be introduced to increase the degree of deacetylation to produce the final product of chitosan. The product can be tested with acetic acid, in which the solubility of the resulting product in acetic acid will indicate a high degree of deacetylation [59].

4. 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 3 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 3. 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 3. 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	Reference
Banana peel (<i>Musa acuminata</i>)	-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	[69]
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	[70]
<i>Brachystegia eurycoma</i> 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	[71]
<i>Brassica spp.</i> seed protein	Pollen grain surface	-6.8 mV	6.5 kDa	-N/A-	-N/A-	95 °C	-N/A-	-N/A-	[29,72]
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 <i>E. coli</i> from dam water	[73]

Table 3. Cont.

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	Reference
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	[2,74]
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 ₂ O (5.5%), CaO (4.2%), Fe ₂ O ₃ (1.5%), SO ₃ and SiO ₂ (0.87%), Al ₂ O ₃ (0.74%), C (0.10%),	-N/A-	-N/A-	-N/A-	[75]
<i>Cassia obtusifolia</i> 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	[76]
<i>Ceratonia siliqua</i> 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-	[29,77]
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	[78–80]
Chitosan extracted from lobster shell (<i>Thenus unimaculatus</i>)	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-	[81,82]
Citrus <i>Limettioides</i> 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-	[75,83]

Table 3. Cont.

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	Reference
<i>C. obtusifolia</i> 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	[84,85]
<i>Cocos nucifera</i> seed protein	Porous structure, clustered, aggregated shapes	-N/A-	5.6 kDa	O-H, N-H	-N/A-	-N/A-	-N/A-	10 g·L ⁻¹ dosage, 96% removal of As(III) in 8 h, 80 rpm and 50 °C	[29,86]
<i>Cucumis melo</i> 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·L ⁻¹ dosage, pH 7, 91% removal of Mn(II) 0.5 g·L ⁻¹ dosage, pH 6.5, 91% removal of Pb(II)	[75,87,88]
<i>Cyamopsis tetragonoloba</i> seed gums	Nanoparticles	-6.66 mV	50–800 kDa	O-H	-N/A-	-N/A-	-N/A-	-N/A-	[29,89]
<i>Dolichos lablab</i> 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·L ⁻¹ dosage, pH 11, 99% removal of turbidity	[29,90]
Garden cress (<i>Lepidium Sativum</i>)	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·L ⁻¹ dosage, pH 5, 99% removal of turbidity from river water	[91]

Table 3. Cont.

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	Reference
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·g ⁻¹ dosage, pH 10, 99% removal of turbidity in surface water and industrial wastewater	[92]
<i>H. esculentus</i>	Compact, cross linkage of molecules	-N/A-	100 kDa	O-H, C-H, C=O	-N/A-	180 °C	36.12 mV	-N/A-	[3,93]
Kenaf crude extract (KCE)	-N/A-	-8.3 mV	-N/A-	-N/A-	-N/A-	-N/A-	-N/A-	100 mg·L ⁻¹ dosage, 85% removal of kaolin, 40 mg·L ⁻¹ , 83% removal of turbidity from river water	[94]
<i>Klebsiella pneumoniae</i>	-N/A-	-N/A-	-N/A-	COO ⁻ , O-H, N-H	C, N, O	-N/A-	-N/A-	pH 7, 40% removal of Cd	[2,95]
<i>Lens culinaris</i>	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·L ⁻¹ dosage, 99% removal of kaolin, 3 min settling time	[96]
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-	[92]

Table 3. Cont.

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	Reference
<i>Maerua decumbent</i>	-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·m ⁻³ dosage, pH 5.56, settling time 52.31 min, 99% removal of turbidity from paint industry wastewater 0.8 kg·m ⁻³ dosage, pH 5.11, settling time 53.53 min, 79% removal of COD from paint industry wastewater	[1]
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·L ⁻¹ dosage, pH 3.01, 97% removal of kaolin	[97]
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-	[75,98]
<i>Moringa oleifera</i>	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·L ⁻¹ dosage, 94% removal of kaolin	[2,94,99,100]
<i>Nirmali</i> seeds	highly porous with reticulated structure	-N/A-	12 kDa	COOH, O-H	-N/A-	-N/A-	-N/A-	1.5 mg·L ⁻¹ dosage, 96% removal of turbidity from surface water	[2,101]

Table 3. Cont.

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	Reference
<i>okra</i>	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·L ⁻¹ dosage, 85% removal of fluoride from hydrofluoric acid synthetic wastewater 20 mg·L ⁻¹ dosage, 94% removal of kaolin, 40 mg·L ⁻¹ dosage, 98% removal of turbidity from river water	[99]
<i>Prosopis spp.</i> 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-	[29,94,102]
Sabdariffa crude extract (SCE)	-N/A-	-6.4 mV	-N/A-	-N/A-	-N/A-	-N/A-	-N/A-	60 mg·L ⁻¹ dosage, 88% removal of kaolin, 40 mg·L ⁻¹ dosage, 96% removal of turbidity from river water	[94]
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·L ⁻¹ dosage, pH 7, 69% removal of turbidity from surface water	[78]
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 <i>Chlorella vulgaris</i>	[2,29,103]

Table 3. Cont.

Natural Coagulant	Surface Morphology	Surface Charge	Molecular Weight	Functional Group	Elemental Property	Thermogravimetry Analysis	Differential Scanning Calorimetry	Performance	Reference
<i>Tamarindus indica</i> 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	[29,104,105]
<i>Telfairia occidentalis</i> 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	[106,107]
<i>T. foenum graecum</i> 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-	[29,108]
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	[109]
<i>Vigna unguiculata</i> 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	[29,106,107, 110]

Note: -N/A- denotes unavailable.

4.1. 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 [111]. Besides, citral is an antimicrobial element that will protect coagulant such as chitosan from microbial damage [112]. 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 [107] 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 [113]. 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 [114]. 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 [2] 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 [115]. Additionally, the high molecular weight allows natural coagulant's chains to stretch sufficiently far from the particle surfaces; thus, favourable for bridging to form [81]. Another study by Muylaert et al. [116] 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 [116]. It had also been well reported that the high molecular weight of natural coagulant would usually predominant in bridging mechanisms. Yin [2] 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 [117]. Such approach is suitable to be used in microalgae harvesting.

4.2. 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 [116]. 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 [116]. Additionally, natural coagulants often perform poorly in harvesting marine microalgae [118]. 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 [116]. 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 [119]. 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 [2]. 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 [3]. These could aid in removing pathogenic bacteria such as *Salmonella paratyphi* that is presented in wastewater due to the leaching of sewage effluents. Thus, phenolic groups provide the $-\text{OH}$ group not only for bridging, but to indirectly inactivate the pathogenic bacteria in the wastewater [3]. 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 [2,120]. Besides, the recent study on biopolymer coagulant showed that 73% (from 320.0 to 88.0 $\text{mg}\cdot\text{L}^{-1}$) of Fe^{3+} reduction and ~36% of COD removal with an addition of 3.20 $\text{mg}\cdot\text{L}^{-1}$ of okra mucilage during the harvesting process [120]. 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^- [2]. 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.

4.3. 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 [121]. 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.

5. 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 [120,122,123]. To illustrate, the plant-based coagulant could be applied on microalgae harvesting at relatively low cost [124]. 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 [125]. 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 [126]. 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 Tables 3 and 4. 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 4 shows the advantages of natural coagulant in microalgae harvesting.

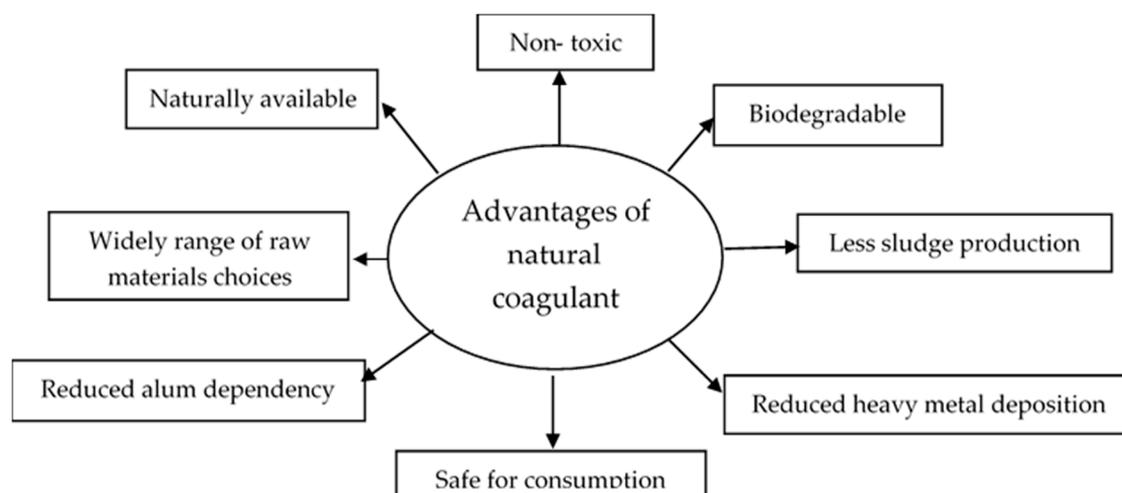


Figure 4. Advantages of utilising natural coagulant in microalgae harvesting.

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 [127]. The study carried out by Tran et al. [128] to harvest *Chlorella vulgaris* with alkyl-grafted chiton $\text{Fe}_3\text{O}_4\text{-SiO}_2$ showed 90% of biomass removal by merely employing $0.013\text{g}\cdot\text{L}^{-1}$ 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 $8\text{mg}\cdot\text{L}^{-1}$ dosage and 96% of harvesting efficiency in 20 min when combining *M. oleifera* with chitosan [129]. Furthermore, 60% of microalgae removal efficiency was achieved with $12\text{mg}\cdot\text{mL}^{-1}$ of *F. indica* extract after 120 min of settling time [130]. 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 4 shows the application of natural coagulants on microalgae harvesting.

Table 4. Application of microalgae harvesting using natural coagulants.

Natural Coagulant	Operating Condition	Performance	Reference
Alkyl-grafted chiton $\text{Fe}_3\text{O}_4\text{-SiO}_2$	$0.013\text{g}\cdot\text{L}^{-1}$ dosage	90% removal of <i>Chlorella vulgaris</i>	[128]
<i>M. oleifera</i>	$8\text{mg}\cdot\text{L}^{-1}$ dosage	76% removal of <i>Chlorella vulgaris</i>	[129]
<i>M.oleifera</i> with chitosan	$8\text{mg}\cdot\text{L}^{-1}$ dosage	96% removal of <i>Chlorella vulgaris</i>	[129]
<i>F. indica</i>	$12\text{mg}\cdot\text{mL}^{-1}$ dosage	60% removal of microalgae	[130]
<i>Pleurotus ostreatus</i> strain HEI-8	pH 3, glucose content $20\text{g}\cdot\text{L}^{-1}$, fungi pelletisation time 7 days, 100 rpm	65% removal of <i>Chlorella</i> sp.	[131]
<i>Citrobacter freundii</i> (No. W4) and <i>Mucor circinelloides</i>	pH 7, glucose concentration $1.47\text{g}\cdot\text{L}^{-1}$	97% removal of <i>Chlorella pyrenoidosa</i>	[132]
Tannin	$11\text{mg}\cdot\text{L}^{-1}$ dosage, pH 5 to 7	97% removal of <i>Chlorella vulgaris</i>	[133]
Tannin	$5\text{mg}\cdot\text{L}^{-1}$ dosage, pH 7	80% removal of <i>Oocystis</i> microalgae	[134]
<i>Eucalyptus globulus</i>	$20\text{mg}\cdot\text{L}^{-1}$ dosage	95% removal of <i>Scenedesmus</i> sp.	[135]
Cassia gum	$80\text{mg}\cdot\text{L}^{-1}$ dosage	93% removal of <i>Chlamydomonas</i> sp.	[136]
Cassia gum	$35\text{mg}\cdot\text{L}^{-1}$ dosage	92% removal of <i>Chlorella</i> sp.	[136]

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.

6. Cost analysis of Natural Coagulants in Microalgae Harvesting

In general, the natural coagulants can be utilised for various applications as demonstrated in Figure 5.

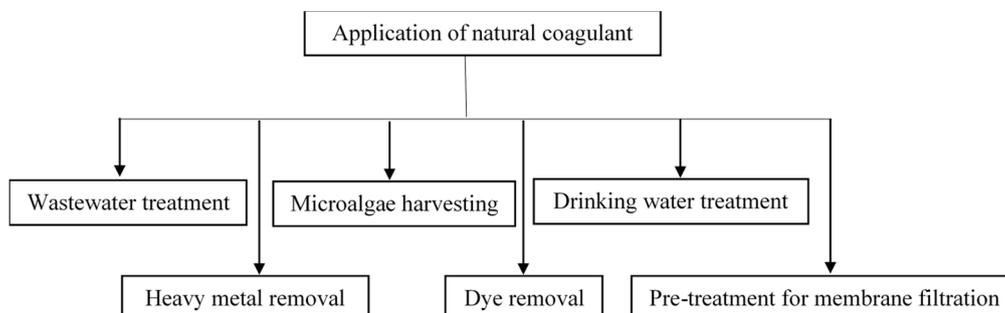


Figure 5. Potential applications of natural coagulants.

For specific instances, natural coagulants reduce suspended solids, traps *E. coli*, reduces turbidity, removes COD, adsorbs heavy metals, harvests microalgae, decolorises dye and others. With regards to the preparation stages, the natural coagulants derived either from plant, animal or microbial feedstock can be facilely produced as opposed to chemical-based coagulant, namely, alum [2]. Moreover, natural coagulants are also more sustainable off late, thus research should be intensified on the exploration of new natural coagulants to substitute the conventional alum. Nonetheless, it is postulated that an abundance of new natural coagulants is yet to be discovered.

On another note, the main drawback of utilising natural coagulant in industries is their low availability for large scale employment [2] as compared with alum. It had been reported by Mubarak et al. [127] on the suitability for large-scale application of natural coagulant and it is limited by the cost of preparation. This has directly led to the necessity of cost assessment on life cycle and cost analysis of different natural coagulants and to compare with alum as depicted in Table 5 [127,129].

Table 5. Comparison of cost analyses between natural coagulants and alum to harvest microalgae [127].

Coagulant	Energy Consumption (Mega Joule per Metric Tons, MJ/MT of Microalgae)	Greenhouse Gas (GHG) Emission (kg CO ₂ eqv/MT of Microalgae)	Cost Analysis (\$/MT)
Chitosan	300	70	9.02
Alum	200	50	0.28
Plant-based coagulant	175	40	0.037

Although the cost analysis by Behera and Balasubramanian [129] showed that the plant-based natural coagulant was relatively cheaper as compared with alum and chitosan in harvesting with a basis of a unit MT of microalgae, it only covered the cost of the harvesting process. As a matter of fact, the extraction process is generally time-extensive. A good illustration has been presented in Figure 2, in which the various processes are involved during the extraction of natural coagulants such as the addition of acid, the aid of equipment as well as the refining tertiary stage. Besides, the extraction is largely confined in the laboratory scale, which may not be feasible in terms of process scalability for industrial applications. An evaluation and approval from the local governing bodies are also part of concerns to commercialise the natural coagulants in the industry. Moreover, the overall costing must take into account of the stringent screenings and documentations that are needed to ensure product compliance to the respective standards [16]. Though the presented costing values in Table 5 are exclusively limited to only the harvesting process, the commercialisation and regulatory authorities

are new inputs and cost-effective extraction techniques are vital to scale up the application of natural coagulants in the future. Further, researchers should pay close attention to the costing of natural coagulants from the primary stage, which involves the plant, animal and microbe selection to the final end product, i.e., plant-based, animal-based and microbe-based coagulant. Exploration in further research should be focused on economical extraction technology of natural coagulant to replace the alum in the near future.

7. Potentially New Natural Coagulant Yet to Be Exploited and Applied

To summarise, our study provides an additional list of potential new natural coagulant to be studied in the future. To recall, mucilage is a criterion of selection for new natural coagulant and it attributed to COO^- and OH^- functional groups, which are mainly associated with the flocculating activity. Besides, it has been espoused in Section 4.2 that galacturonic acid in mucilage is the active component that aids the coagulation–flocculation. Therefore, the most reliable method to predetermine the potentially new natural coagulant is to study the chemical composition, galacturonic acid, in each natural coagulant. A previous study [137] noted that pectic acid (polygalacturonic acid) extracted from sugar beet pectin comprises approximately 68 percent of the galacturonic acid. Moreover, pectic acid from flax pectin was found out to be made up of 61 percent of galacturonic acid, and the pectic acid from orange peelings was composed of 73.7 percent of galacturonic acid [137]. To sum up, natural coagulant often been extracted from materials with galacturonic acid as its chemical composition. Thus, the chemical components, galacturonic acid in mucilage is a point of interest in the selection of potentially new natural coagulant. Table 6 shows the potentially new natural coagulant.

Table 6. Potentially new natural coagulant.

Possible Natural Coagulant	Scientific Name	Reference
Cowpea	<i>Vigna unguiculata</i>	[138]
Chia seeds	<i>Salvia hispanica</i> L.	[139]
Rockcress	<i>Arabidopsis thaliana</i>	[140]
Quince seed	<i>Cydonia oblonga</i>	[141]
Jujube	<i>Ziziphus mauritiana</i> Lam	[142]
Seashore mallow	<i>Kosteletzkya virginica</i>	[143]
Watershield	<i>Brasenia schreberi</i>	[144]
Beet root	<i>Alyssum homolocarpum</i>	[145]
Levant wormseed	<i>Artemisia sphaerocephala</i>	[146]
Fenugreek seed	<i>Trigonella foenum-graecum</i> L.	[147]
Cress seed	<i>Lepidium sativum</i>	[148]

8. Conclusions

The usage of natural coagulants derived from plant, animal and microbial sources in industry is a trend of sustainable environmental development in the 21st century. Particularly, natural coagulant should be given priority in microalgae harvesting as it is highly effectual in flocculating activity and will not leave a negative impact to the end product due to its biodegradability.

On the other hand, the extraction processes differ for each type of natural coagulant. A comprehensive review is conducted to explain and justify the necessity to carry out each sub-step in the extraction process of natural coagulant. This information is useful in the exploration of new natural coagulant in the future.

Furthermore, the characterisation of natural coagulants is vital in enhancing their flocculating activity. The modification such as grafting could be used to increase or decrease the zeta potential and to provide more functional groups for attachment, which fundamentally enhancing the flocculating activity of natural coagulant. Moreover, molecular weight determines the coagulation mechanism of natural coagulant, for example, high molecular weight of natural coagulant (when they are more than 1×10^6 kDa) would usually predominant in bridging mechanisms. The functional group of natural

coagulant identifies the effective groups, which help in the coagulation–flocculation process, typically O-H and C-H groups.

The applications of natural coagulant in the industry are summarized in this review, for instance, wastewater and drinking water treatment, heavy metal and dye removal and pretreatment for membrane filtration and microalgae harvesting. In view of the current studies, there is no doubt that the application of natural coagulant in microalgae harvesting will play a significant role in upscaling for mass production. To illustrate this, chitosan requires only $0.013 \text{ g}\cdot\text{g}^{-1}$ algae dosage to remove 90% of *C. vulgaris* as compared to $0.101 \text{ g}\cdot\text{g}^{-1}$ of polyaluminium chloride to remove 93% of algae [128] or $30 \text{ mg}\cdot\text{L}^{-1}$ of alum (aluminium sulphate) to remove 95% of *C. vulgaris* [149]. Importantly, using chitosan as coagulant does not inhibit the downstream process of transesterification biodiesel production on both the enzyme and chemical catalysed while other coagulants do [128]. Furthermore, tannin requires $11 \text{ mg}\cdot\text{L}^{-1}$ dosage to remove 97% of *C. vulgaris* [133], and *M. oleifera* with chitosan requires $8 \text{ mg}\cdot\text{L}^{-1}$ dosage to remove 96% removal of *C. vulgaris* [129]. These results show that natural coagulant is efficient in harvesting algae with a relatively lower dosage than alum. Additionally, natural coagulant deserves an attention on the harvesting of microalgae that produced DHA oil due to its non-toxic and non-chemical nature.

As noted in Section 2, the extractions of plant-based coagulants require specialised knowledge in identifying the potential plants as a coagulant and require performing detailed extraction stages, which are time-consuming. Common problems are also encountered in the preparation of animal- and microbial-based coagulants. At this point, the mass production of natural coagulants is still economically infeasible due to its complexity in bulk processing, low-volume in market demands and lack of supportive regulation that stipulates the quality of the natural coagulant extracts [2]. In view of this, the natural coagulant is currently restricted to small-scale usage and academic research, but it has the potential, especially for bulk microalgae harvesting in industries. Moreover, optimisation of natural coagulant based on their respective characteristic will further enhance its efficiency in coagulation and the result will be significant regarding mass harvesting of microalgae. The key effort of this paper includes the production of economical and sustainable natural–organic coagulants in the future.

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