

A review on development and application of plant-based bio-flocculants and grafted bio-flocculants

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Abstract

Flocculation is extensively employed for clarification through sedimentation. Application of eco-friendly plant-based bio-flocculants in wastewater treatment has attracted significant attention lately with high removal capability in terms of solids, turbidity, colour and dye. However, moderate flocculating property and short shelf life restrict their development. In order to enhance the flocculating ability, natural polysaccharides derived from plants are chemically modified by inclusion of synthetic, non-biodegradable monomers (e.g. acrylamide) onto their backbone to produce grafted bio-flocculants. This review is aimed to provide an overview of the development and flocculating efficiencies of plant-based bio-flocculants and grafted bio-flocculants for the first time. Furthermore, the processing methods, flocculation mechanism and the current challenges are discussed. All the reported studies about plant-derived bio-flocculants are conducted under lab-scale conditions in wastewater treatment. Hence, the possibility to apply natural bio-flocculants in food and beverage, mineral, paper and pulp, oleo-chemical and biodiesel industries is discussed and evaluated.

Keywords: plant-based bio-flocculants; grafted copolymers; flocculation; clarification; polysaccharides; biodegradable

Abbreviations

COD: chemical oxygen demand

SS: suspended solids

LDS: light diffraction scattering

TDS: total dissolved solids

1. Introduction

Flocculation is one of the important separation processes that are extensively employed in portable water, domestic or industrial wastewater treatment. Various types of flocculants have been developed and marketed for the removal of environmental concerned parameters such as suspended and dissolved solids, turbidity, chemical oxygen demand (COD), colour and dye

through sedimentation. They have been applied in diverse range of wastewaters such as food and beverages, paper manufacturing, agricultural production, dyes or textile, municipal and others.⁵ One of the leading manufacturers of flocculants (BASF: Badische Anilin- und Soda-Fabrik) reported that the global market for cationic polyacrylamide flocculants is worth around € 1.0 billion and is growing at 4% to 5% per year.⁶ Through addition of flocculant(s), finely suspended or dispersed particles are aggregated together to form flocs into the size for speedy sedimentation and clarification. The conventional flocculants used in treatment of water and industrial effluents can be classified into two categories depending on the chemical compositions which are inorganic flocculants and organic polymeric flocculants.

Inorganic flocculants (salts of multivalent metals like aluminium and iron) are commonly being used due to its low cost, ease of use and availability. Nevertheless, their usage has been reduced and controlled due to some disadvantages such as large amount is required for efficient flocculation, highly sensitive to pH, applicable to only a few dispersed systems, and inefficient towards very fine particles.⁷ Acidic or alkaline solutions are always needed to alter the pH of the wastewater in order to achieve its isoelectric point and formation of complex metal hydroxide for precipitation or sedimentation.⁸ Generally, inorganic flocculants are cationic-based and the impurity particles are negatively charged. The flocculation mechanism involved is either charge neutralisation or patching where the flocs formed with the mechanism as shown in Figures 1 and 2 are loosely packed and settled slowly.⁹ Besides, its application has caused problems of increased metal concentration or residual aluminium in treated water which may have human health implications, and produces large quantity of sludge which disposal itself is another problem.^{7, 10}

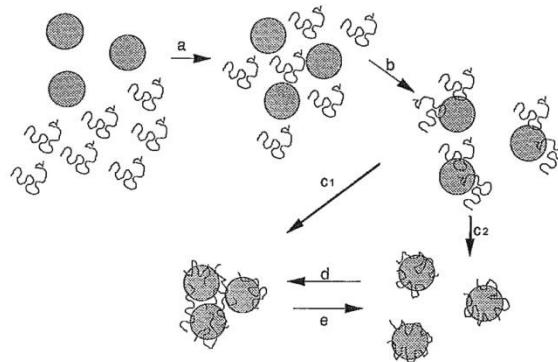


Figure 1. Action of charge neutralisation of cationic flocculant with the particle surface¹¹

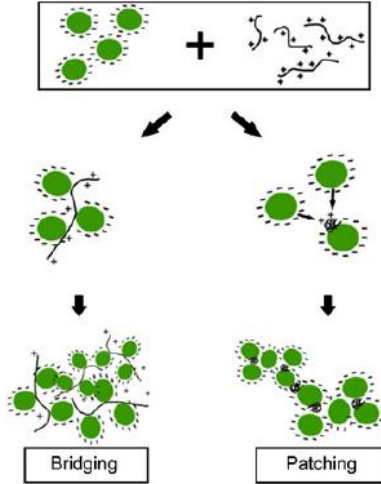


Figure 2. Flocculation mechanisms of bridging and patching¹²

Organic polymeric flocculants such as polyacrylamide have become very important in wastewater treatment due to its greatest advantage with remarkable ability to flocculate even when added in small quantities (ppm).⁷ High molecular weight polymers with long chain are absorbed on particles as shown in Figure 2 with loops and tails extending into solution and give the possibility of attachment of these ‘dangling’ polymer segments onto other particles, thus ‘bridging’ particles together.¹³ Hence, the flocs formed are bigger, stronger and denser with good settling characteristics.¹⁴ In addition, they are easy to handle, immediately soluble in aqueous systems, not sensitive to pH and produce lower sludge volume.¹⁰ However, the potential problems associated with their use are lack of biodegradability and dispersion of monomers or residual polymers in water that may represent a health hazard.^{10, 13, 15} Sludge formed at water treatment plants with polymeric flocculants has a limited potential for recycling due to the non-biodegradability of synthetic polymers.¹

With increasing awareness of potential harms caused by chemical flocculants and implementation of more stringent environmental regulations, some countries (e.g. Japan, Switzerland and France) have started to strictly control its usage in drinking water treatment and food-related processing.^{13, 16} Researchers are trying to discover high efficient and eco-friendly bio-flocculants with the aim to replace the conventional flocculants. Biopolymers based flocculants have attracted wide interest from researchers because they have the advantages of biodegradability, non-toxic and easily available from reproducible agricultural resources.^{10, 14, 17}

Natural organic flocculants which are based on natural polymers or polysaccharides like starch, cellulose, chitosan, natural gums, mucilage and etc. have been investigated for their flocculating properties in wastewater treatment. Chitosan (amino-polysaccharides) has received a great deal of attention in the last decades in water treatment processes for the removal of particulate inorganic or organic suspensions and dissolved organic substances.¹⁰ Starch itself may be used as flocculant; however, its flocculation efficiency is low. As a result, starch is generally modified in order to obtain products with good flocculation efficiency and has been applied in treating wastewater^{18, 19} and papermaking industry.²⁰ Sodium carboxymethylcellulose

(CMCNa) is produced by chemical modification of cellulose and has been tested as eco-friendly flocculants for drinking water treatment.²¹ Natural gums, such as guar gum and xanthan gum have been studied extensively as an effective flocculants over a wide range of pH and ionic strengths for treatment of wastewater from various industries.^{22, 23}

The plant-based bio-flocculants contain natural polysaccharides which are suspected to exhibit excellent selectivity towards aromatic compounds and metals, thus efficient in the removal of pollutants from wastewater.²⁴ However, it was reported that their feasibility is restricted by moderate flocculating property and short shelf life.²⁵ In recent years, grafted bio-flocculants are developed and claimed to have remarkable flocculating ability and biodegradability. As shown in Figure 3, natural bio-polymers are covalently bonded (modified) by inclusion of synthetic monomers (e.g. acrylamide) onto their backbone to synthesise the high molecular weight grafted copolymers that exhibit improved flocculating properties.²⁶

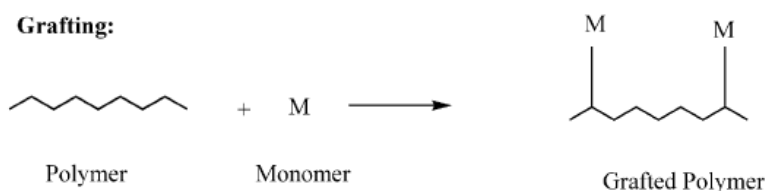


Figure 3. Schematic representation of grafting method in polymer modification²⁷

The rising concern of environmental pollution problems and health-concerning issues causes the utilisation of bio-flocculants derived from natural sources as an important progress in sustainable environmental technology. In this review, the development and flocculating abilities of plant-based bio-flocculants and grafted bio-flocculants is reported for the first time. The preparation methods of plant-based bio-flocculants and the grafting methods of grafted copolymers are presented and reviewed. Their flocculating effectiveness in wastewater treatment and the relevant flocculating mechanisms are investigated in detail. This review is aimed to provide a clear and comprehensive conspectus about the research progress in developing plant-based bio-flocculants and grafted bio-flocculants, the current challenges and future perspectives, and the potential application of plant-based bio-flocculants in diverse industries.

2. Plant-based bio-flocculants

Natural plant-based bio-flocculants emerge as an attractive alternative to polymeric flocculants and their applications in wastewater treatment has become increasingly essential, in light of their biodegradability, non-toxic, widely available from renewable resources, environmental friendly processing and have no negative impact on the environment. The applications of plant-derived bio-polymers for treatment of various types of wastewater have been discovered and reported. Plant-based bio-flocculants derived from some plant species (*Hibiscus/Abelmoschus esculentus*, *Malva sylvestris*, *Plantago psyllium*, *Plantago ovata*, *Tamarindus indica*, and *Trigonella foenum-graecum*) have shown promising results with respect to the treatment of biological effluent,

landfill leachate, dye-containing wastewater, textile wastewater, tannery effluent, and sewage effluent.^{1, 3, 4, 28}

2.1 Plant materials and bio-flocculants preparation methods

Table 1 shows six different types of plants which have been investigated of their flocculating properties in the treatment of synthetic or genuine wastewater. It is discovered that all plants being studied for flocculants production have one similarity. All of them have mucilaginous texture with polysaccharides as the main components and they are having neutral pH in nature. Mucilage is plant hydrocolloids that have viscous colloidal dispersion properties in water. They are heterogeneous in composition and are typically polysaccharide complexes formed from the sugars of different monosaccharides, including arabinose, galactose, glucose, mannose, xylose, rhamnose and uronic acid units.²⁹⁻³¹ It is predicted that some of the active ingredients in the mucilage are responsible for the flocculating property. Therefore, extraction becomes the essential step to isolate the active components that exhibit the flocculating activity from the plants. However, the investigation of active constituents that are corresponding with the flocculating ability is limited.

Table 1. Plants that have been investigated of their flocculating properties

Plant species		Charge	pH	Solubility in water	Active ingredients	Extraction method	Extracted plant part	References
Scientific name	Common name							
<i>Hibiscus / Abelmoschus esculentus</i>	Okra / lady finger	Anionic	5.2 to 8	Soluble in cold water	L-rhamnose, D-galactose and L-galacturonic acid	Solvent extraction and precipitation, drying and grinding	Seedpods	1, 32-37
<i>Malva sylvestris</i>	Mallow	-	6.5 to 7	-	-	Drying and grinding	Seedpods and lobes	1
<i>Plantago psyllium</i>	Psyllium	Anionic	7.11 to 7.84	Soluble in cold water	L-arabinose, D-xylose and D-galacturonic acid	Solvent extraction and precipitation	Seed husk	2, 3, 38
<i>Plantago ovata</i>	Isabgol	Anionic	-	-	-	Drying and grinding	Seed husk	28
<i>Tamarindus indica</i>	Tamarind	-	-	Soluble in cold water	D-galactose, D-glucose and D-xylose	Solvent extraction and precipitation	Seeds	4
<i>Trigonella foenum-graecum</i>	Fenugreek	Neutral	7.73 to 8.62	Partially soluble	D-galactose and D-mannose	Solvent extraction and precipitation	Seeds	37, 39, 40

There are two methods for the production of plant-derived bio-flocculants which have been reported thus far, which are (i) solvent extraction and precipitation^{2, 3, 33, 36-41} and (ii) drying and grinding.^{1, 28} The detailed procedures for each method are illustrated in Figure 4. The methodologies used to produce the bio-flocculants are environmental-friendly, no toxic chemical used, easy and simple. As listed in Table 1, the solvent extraction and precipitation method has been applied for extraction of bio-flocculants from Okra, Psyllium, Tamarind and Fenugreek. The cleaned plant materials were extracted with distilled water for overnight and the filtered mucilaginous extract was then precipitated by using alcohol. The drying and grinding method has been used for preparation of bio-flocculants from Mallow, Okra, and Isabgol. The cleaned materials were dried at high temperature, and then grounded and sieved to obtain the bio-flocculants. It was discovered that the bio-flocculants obtained had lower flocculating activity. Low removal efficiency of COD, color and suspended solids (SS) at 17, 27 and 41% respectively

was reported when it was used as flocculant in treatment of landfill leachate with direct flocculation process.²⁸ They were found to be more effective in coagulation-flocculation process where coagulant was added before bio-flocculant. On the other hand, the bio-flocculants that are prepared with solvent extraction and precipitation displayed excellent flocculating ability in the treatment of wastewater with direct flocculation process where no coagulant and pH adjustment are required. This finding indicated that extraction step is closely related with flocculating efficiency and plays the major role to extract the active constituents with high flocculating activity from the plant materials.

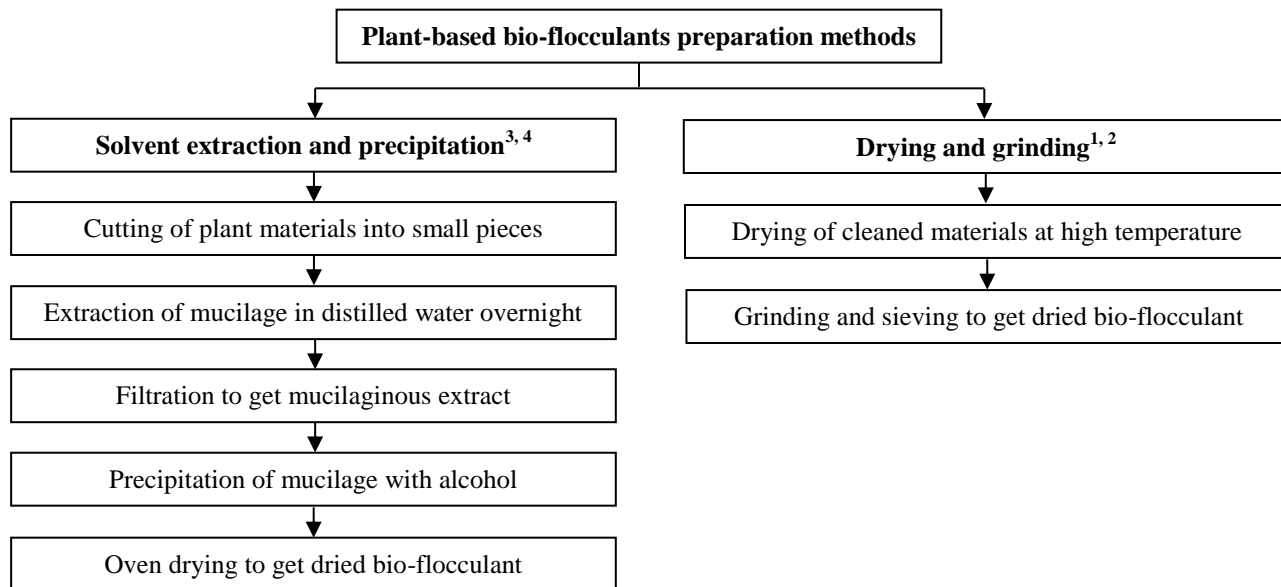


Figure 4. Preparation methods of plant-based bio-flocculants

Up to date, all the studies that investigated the flocculating property of bio-flocculants only paid attention to the flocculation process and analysed the effects of flocculant dose, contact time and pH on the flocculation efficiency.^{2, 3, 40} There is no published study that investigates the relationship between extraction and flocculation. It is very important to relate the extraction methods and conditions with the flocculating activity, evaluate the extraction parameters that may degrade the flocculating efficiency of the products, and optimise the extraction conditions in order to produce the most efficient bio-flocculants that are comparable to commercial flocculants in terms of cost and flocculating efficiency.

2.2 Flocculation efficiency

Jar test⁴² is used to evaluate the flocculating abilities of the plant-based bio-flocculants and optimise the flocculation process in most studies. The identified usage of natural flocculants and their technical viability for industrial wastewater are currently limited to only academic research. Their flocculating performance reported in literature has been compiled and summarised in Table 2. The findings indicated their good flocculating potential for various types of industrial

wastewater treatment. Some of them are effective in low concentrations and comparable to synthetic flocculants in terms of treatment efficiency.^{33, 40} Fenugreek⁴⁰ and Okra³³ mucilage were proven to be as effective as commercial flocculant (polyacrylamide) in treatment of tannery effluent and sewage wastewater.

Table 2. Flocculating efficiencies of plant-based bio-flocculants

Plant-based bio-flocculants and its preparation method	Treated wastewater	Sedimentation period (minutes)	Optimum pH	Optimum coagulant dose (mg/L)	Optimum bio-flocculant dose (mg/L)	Flocculation efficiency		Reference
						Types of removal	% removal	
Isabgol (drying and grinding)	Semi-aerobic landfill leachate	120	6.5 to 7.5	7200	400	COD	64	28
						Colour	90	
						SS	96	
Psyllium (extraction and precipitation)	Golden yellow dye and reactive black dye	10	4 and 7	-	10	Dye	71.4 and 35	3
	Textile	60	4	-	1.6	SS	92.4	2
	Sewage and tannery	60	7	-	1.2	TDS	68.76	38
					SS	94.69 and 87.03		
Tamarind (extraction and precipitation)	Golden yellow dye and direct fast scarlet dye	60	7	-	10 and 15	Dye	60 and 25	4
	Azo dyes, basic dyes and reactive dyes	10	6, 9.2 and 1	-	10, 10 and 15	Dye	36.43, 38.96 and 52.13	41
Fenugreek (extraction and precipitation)	Tannery	60	7	-	0.08	SS	85	40
	Sewage	60	9.2	-	0.16	SS	97.15	39
						TDS	19.9	
Okra (extraction and precipitation)	Tannery	60	9.2	-	0.04	SS	95	36
	Sewage	60	4	-	0.12	TDS	69	33
						SS	86.86	
Okra and Fenugreek (extraction and precipitation)	Textile	60	9.2	-	0.8 and 0.04	TDS	>95	43
						SS	98 and 94.2	
						Colour	31.6 and 43.7	
Mallow and okra (drying and grinding)	Synthetic (kaolin)	10, 20, 30	6.2 and 6	8.6	12 and 5	Turbidity	53.47 and 6	1
	Biologically-treated effluent		-	171.1	62.5 and 2.5		97.4 and 97.3	
							67 and 74	

As reported in the previous section, bio-flocculants obtained with drying and grinding exhibit lower flocculating efficiency. These bio-flocculants must be coupled with usage of coagulant in coagulation-flocculation process. Table 2 shows that the Isabgol husk prepared with drying and grinding was effective as coagulant aid for the treatment of landfill leachate with poly-aluminium chloride (PACl) as the coagulant.²⁸ However, unsatisfactory results were obtained when it was used as primary coagulant aid without coagulant due to low surface charge. Another recent study showed that the dried and grinded mallow and okra bio-flocculants were efficient in removing turbidity from synthetic kaolin suspension and biologically-treated effluent when aluminium sulphate was used as the coagulant.¹ On the other hand, the bio-flocculants extracted with water as the solvent display remarkable flocculating performance in direct flocculation process. High removal efficiency of solids either in suspended (SS) or dissolved forms (TDS), dye, turbidity and colour was achieved by using low concentration of bio-flocculant dosage. For instance, 95% removal of SS in treatment of tannery wastewater³⁶ and >95% removal of TDS in treatment of sewage effluent³³ were attained with 0.04 mg/L and 0.12 mg/L of okra bio-flocculant. Mallow and okra bio-flocculants were reported to have high efficiency in turbidity removal at 97% in treatment of synthetic (kaolin) wastewater.¹ In other paper, dye removal as

high as 71%³ and colour removal at 53%⁴³ were achieved by using psyllium and okra bio-flocculants respectively.

As shown in Table 2, long sedimentation period (60 and 120 minutes) was reported in some literature. It is postulated that the flocs formed are weaker and smaller in size and thus longer settling time is required. In the study of *Tamarindus indica* (Tamarind) as bio-flocculant, it was not suggested as an effective flocculant for the removal of vat (golden yellow) and direct (direct fast scarlet) dyes from textile wastewater because of unsatisfied dye removal after a long period of contact time.⁴ In most of the reported studies, the suitable pH range was neutral for maximum flocculating efficiency of bio-flocculants. Some were reported to be workable in acidic or alkali condition depending on the type and characteristics of treated wastewater.

2.3 Flocculation mechanism

The most common mechanisms of flocculation include charge neutralisation, electrostatic patch and polymer bridging.¹³ The flocculation mechanism of charge neutralisation is only applicable when the colloid suspended particles and the added flocculants are of opposite charge.¹³ In many cases, impurity particles are negatively charged. As shown in Table 1, the ionic charges of bio-flocculants are anionic for Okra, Psyllium and Isabgol, neutral for Fenugreek and unknown for Mallow and Tamarind. Since most of the bio-flocculants are verified to be either anionic or neutral, it is presumed that charge neutralisation is not the responsible mechanism. Thus, the most probable mechanism that happened between plant-derived bio-flocculants with particulate matter in effluent is polymer bridging where the bio-polymers serve as a bridge based on particle-polymer-particle complex formation. The bridging mechanism involved in bio-flocculation as shown in Figure 5 has been reported in a study by using one flocculating microalgae to concentrate the non-flocculating microalgae of interest.¹²

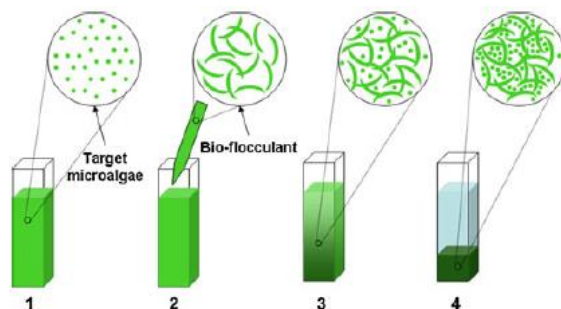


Figure 5. Bridging mechanism in bio-flocculation of microalgae¹²

In order to be effective in destabilisation, a bio-polymer molecule must contain chemical groups (free hydroxyl group), which is the possible binding sites that can interact with sites on the surface of the colloidal particles. When a bio-polymer molecule comes into contact with a colloidal particle, some of these groups adsorb at the particle surface, leaving the remainder of the molecule extending out into the solution. If a second particle with some vacant adsorption sites contacts with these extended segments, attachment will occur. A particle-polymer-particle

complex is thus formed in which bio-polymer serves as a bridge.⁴¹ Polymer bridging has been proposed as the plausible mechanism for flocculation behaviour in treatment of textile wastewater with *Plantago psyllium* mucilage³ and *Tamarindus indica* mucilage.⁴ For other bio-flocculants, the underlying mechanism has not been predicted or reported up to date.

In some studies, X-ray diffractograms were used to observe and postulate the possible mechanism underlying the flocculating property of natural polymers.^{2, 33, 38-40} However, X-ray diffraction patterns did not give any specific evidence for the mechanism of flocculation, but it suggested the interaction of the solid waste with the mucilage. In those studies,^{2, 33, 38-40} the formation of different crystal types were observed after the solid waste was treated with polysaccharide, and this indicated the change in the nature of the crystalline waste material in the wastewater during the flocculation process. This may be due to the interactions between free hydroxyls groups of the polysaccharide and the contents of the wastewater.

As the chemistry of coagulation and flocculation is primarily depend on the electrical properties, analysis of zeta potential was used as a measurement of the magnitude of electrical charge surrounding the colloidal particles and explained the removal mechanisms of the flocculation process.²⁸ Through measurement of zeta potential, the ionic charge of the bio-flocculants and the surface charge of the suspended particles could be defined and this information is useful to predict the plausible flocculation mechanism. Recent studies showed that light diffraction scattering (LDS) is a useful technique to monitor the dynamics of flocculation and used as a tool to evaluate different types of flocculation mechanism depending on the flocculants characteristics.^{44, 45} It enabled an evaluation of the effects of charge density of the flocculant on the fractal dimension of the flocs which serves as the measurement on the compactness of the aggregates. As a summary, by combining the measurement of zeta potential and LDS technique, performance of the bio-flocculants during flocculation could be monitored. The flocculation kinetics and mechanism of the bio-flocculants could then be determined.

2.4 Current challenges and future perspectives

Even though most of the studies have proven that the bio-flocculants were workable and effective for treatment of various types of wastewaters, all these research were carried out in laboratory scale and only tested in treatment of wastewaters. There are many factors that restrict its development and application in industry. The four major problems including sensitivity of bio-products to preparation process, fast degradation with time, moderate flocculating efficiency and higher cost compared to commercial flocculants.

The functional properties of the hydrocolloid mucilage are sensitive to the preparation methods and could be altered by the drying processes to great extent.⁴⁶ In addition, the chemical composition and molecular structure of hydrocolloids often depend on the source, extraction methods and any further processing conditions.³⁰ For instance, the rheological properties and viscoelastic behaviour of natural plant gums depend on the method and condition of extraction, purification, drying and further modification processes.⁴⁷ Thus, more investigation concerning the processing methods and conditions (preparation, extraction, purification, drying and storage)

of bio-flocculants is strongly important because it will determine the quality and stability of bio-flocculants.

Bio-polymers face degradation of products with time and processing parameters or conditions.²⁹ Fresh mucilage is susceptible to microbial attack due to its high water activity and composition, and reducing its shelf life to a few days at room temperature.⁴⁸ In addition, the flocs tend to lose stability and strength with time because of their biodegradability.⁴⁹ Materials with reduced moisture content will resist germination under favourable conditions, thus prolonging the storage life. Therefore, drying process is of vital importance to produce high quality bio-flocculants where the desired active constituents with flocculating property could be well preserved and the storage period could be extended.

Some natural flocculants are moderately effective and are needed in huge dosage compared to synthetic flocculants due to their relatively lower molecular weight and shorter shelf life.²⁵ Hence, optimisation study of processing, extraction and drying conditions is highly important to get the optimum conditions that can produce the bio-flocculants with maximum yield and flocculating ability.

Another significant drawback of using bio-flocculants is the processing and production cost which is higher than conventional flocculants. Yet, this drawback can be overcome due to the major importance of their applications in food and other industries, allowing for a price premium products, and its substantial benefit to environment and human health. The phytonutrients associated in the bio-flocculants will further enhance the quality of the products too.

Nonetheless, the future development of cost-effective and environmental-friendly plant based bio-flocculants that exhibit high flocculating ability as an attractive alternative to replace commercial flocculants in food, cosmetic, pharmaceutical and other industries is highly possible. Intensive research efforts related to bio-flocculants should continue and in-line with environment and health protection.

2.5 Possible application of plant-based bio-flocculants in different industries

To date, there is no detailed study about the applicability or feasibility of plant-derived bio-flocculants in other industries apart from wastewater treatment. Their application studies are only at preliminary level and conducted on laboratory basis. Exploration of application boundaries to new areas of industrial interest instead of focusing on downstream processes (wastewater treatment) is highly important to increase the market need of this product. In fact, there are many processing industries utilizing clarification or flocculation process in the manufacturing of certain products. The clarification or flocculation method or agent used in different industries are summarised in Table 3.

Table 3. Clarification/flocculation process in different industries

Application area		Separated components	Clarification/flocculation method/agent	References
Beverage industry	Clarification of wine	Iron compounds, suspended solids, proteins, dead yeast cells	Activated carbon, gelatin, albumen, bentonite	50-53
	Clarification of vinegar	Iron compounds, suspended solids, proteins	Activated carbon, gelatin, albumen	50, 51
	Clarification of fruit juices	Suspended particles, proteins, polyphenols, pectins, carbohydrates	Gelatin, bentonite, kieselsol, silica sol, polyvinylpyrrolidone (PVPP) or combination of these compounds, chitosan	54-58
	Clarification of dates extract	Non-soluble matter, colouring matter and semi-soluble (e.g. pectin) material	Activated carbon, bentonite, PVPP	59, 60
	Clarification of sugar cane juice	Organic and inorganic constituents, suspended solids	Lime, bentonite, activated carbon, polyacrylamide polymer	61-64
Food industry	Recovery of muscle proteins from fish processing by-products	Bones, scales, skin, fats	Isoelectric solubilisation/precipitation, polymeric flocculants (anionic, non-ionic and cationic)	65, 66
	Recovery of muscle proteins from meat processing by-products	Bones, scales, skin, fats	Isoelectric solubilisation/precipitation, mechanical deboning	67-69
Mineral industry	Clarification of kaolin slurry	Discolouring contaminants (e.g. titanium, iron), organic and inorganic carbon, impurity clay minerals	Anionic polymeric flocculants (e.g. polyvinyl alcohol, polyacrylamide)	70-72
Paper industry	Retention of fine matters during papermaking	Cellulose fines, fibre fines and fillers	Polyelectrolytes flocculants (e.g. polyacrylamides, poly-aluminium chloride, polyethylene oxide), modified starches, chitosan	20, 44, 73-77
Oleo-chemical or biodiesel industry	Clarification of crude glycerol	Methanol, inorganic salt (catalyst residue), free fatty acids, lipids, unreacted mono- or di- or triglycerides, non-glycerol organic matters	Chemical treatments, physical treatments (gravitational settling, centrifugation, evaporation, distillation), micro and ultra-filtration	78-83

Consumers are becoming more health conscious, increasingly aware of what they consume and concern of the nutritional value of food materials and ingredients. Safe and healthy products and environmental-friendly processing are much preferred and will be the primary concern in many industries. Many of the conventional clarification and flocculation methods listed in Table 3 have its own drawbacks. It has become necessary to study a new clarification or flocculation method/agent with the objective of securing a safe and high quality product. In this respect, plant-based bio-flocculants may emerge as an attractive option to alleviate the problems mentioned above; and at the same time enhance the nutritional value of the products. The background and detailed explanation for natural bio-flocculants could be a suitable alternative to the current flocculation/clarification methods in food and beverage, mineral, paper-making, oleo-chemical and biodiesel industries are attached in Supporting Information.

Plant-based bio-flocculants have been proven to remove impurities, turbidity, organic and inorganic loads, suspended and dissolved solids effectively. Besides, natural water-soluble polysaccharides have the capability of flocculating small particles.³⁸ The long chain of polysaccharides has the ability to bind or bridge different components and result in efficient removal of undesirable compounds. Okra,³⁶ Fenugreek⁴⁰ and Psyllium³⁸ bio-flocculants could remove the proteinaceous matter effectively which surrounds the colloidal particles and the

metallic ions in tannery effluent. In addition, they have been verified to have the ability to remove or reduce the inorganic and organic solids, metals, fibres and toxic pollutants from textile and sewage wastewaters.^{2, 39} Thus, they may exhibit the capability to clarify the juices extract, flocculate and recover the muscle proteins from seafood and meat processing by-products and improve the retention of fibres and fillers in papermaking industry.

A study showed that okra and mallow bio-flocculants exhibited the flocculating ability to remove the turbidity from kaolin suspension solution effectively.¹ Therefore, plant-based bio-flocculant may be applied to produce a high brightness and whiteness coating clay which contains a minimum amount of undesirable residual chemicals particularly for application in cosmetic and pharmaceutical industry.

In oleo-chemical industry, the use of natural bio-flocculants is postulated to have the advantages of simple process with reduced number of operations, lower purification time, lower treatment cost and mild conditions of treatment with low temperature and pressure. By using natural bio-flocculants, strong and dense flocs could be formed in short time. After sedimentation, the formed flocs are easily separated through sedimentation. Since no chemical is used in the purification process, the purified glycerol could be applied safely for a range of application in pharmaceutical or cosmetic industries. It is predicted that the bio-flocculants may consists of adsorption sites (e.g. free hydroxyl groups and hydrogen bonding sites) that have strong affinity to remove the undesirable matters or components from crude glycerol.

3. Plant-based grafted bio-flocculants

In recent years, chemical modification of natural macromolecules, especially polysaccharides, has received considerable interest to improve their flocculating properties.^{84, 85} Such modification is done to overcome the drawbacks in term of moderate flocculation performance, uncontrolled biodegradability, and varying efficiency due to different processing conditions.⁸⁶ The biodegradability of natural polysaccharides needs to be suitably controlled⁸⁷ to prolong the shelf life and improve the flocculating ability. On the other hand, synthetic flocculants are highly effective and have long shelf life, but they are non-biodegradable and toxic to environment.^{17, 88} The improvement in the properties of natural and synthetic polymers can be performed by developing grafted copolymers.⁸⁹

Graft copolymerisation is aimed to obtain novel tailor-made polymer with the best properties of both groups. It has been proven that efficient, reasonably shear stable and eco-friendly flocculants can be developed by grafting synthetic polymers branches onto the rigid backbone of natural ones.⁸⁹ Some studies showed that acrylamide-grafted natural polymers, such as amylopectin, guar gum and xanthan gum, starch, and sodium alginate find extensive application as flocculants.^{90, 91} Recently, the common polymers such as polyacrylamide have been grafted onto the backbone of plant-based bio-polymers (*Plantago ovata*, *Plantago psyllium* and *Tamarindus indica*) and showed significant improved flocculating properties compared to ungrafted natural polysaccharides.⁹²⁻⁹⁴ The flocculating performance of all the plant-based grafted bio-flocculants reported in literature is summarised in Table 4.

Table 4. Flocculating efficiencies of plant-based grafted bio-flocculants

Plant-based grafted bio-flocculant	Grafting method	Treated wastewater	Sedimentation time	Optimum dose (ppm)	Flocculation efficiency		Reference
					Type of removal	Results	
Polyacrylamide-grafted-Plantago psyllium (Psy-g-PAM)	Conventional free radical	Tannery and domestic wastewater	1hour	60	SS	>95% and >89%	94
		Textile wastewater	1hour	1.6	SS	>93%	95
						TDS	72%
Polyacrylonitrile-grafted-Plantago psyllium (Psy-g-PAN)	Conventional free radical	Textile effluent	1hour	1.6	SS	94%	96
		Tannery effluent	1hour	1.2	SS	89%	88
						TDS	27%
Polymethacrylic acid-grafted-Plantago psyllium (Psy-g-PMA)	Microwave-assisted	Municipal sewage wastewater	25min	2.5	Turbidity	100 to 12NTU	97
Polyacrylamide-grafted-Plantago ovata	Microwave-initiated	0.25% kaolin suspension	15 min	0.75	Turbidity	59 to 22NTU	93
		1% coal fine suspension			OD	1.5 to 0.25	
Polymethylmethacrylate-grafted-Plantago ovata	Microwave-assisted	0.25% kaolin suspension	15 min	1	Turbidity	185 to 70NTU	98
Polyacrylamide-grafted-Tamarindus indica (Tam-g-PAM)	Conventional free radical	Textile wastewater	10min	5	Azo dye	43%	41
					Basic dye	27-29.6%	
					Reactive dye	26.8-32.3%	
Polyacrylamide-grafted-Tamarind kernel polysaccharide (TKP-g-PAM)	Microwave-assisted	Kaolin suspension Municipal sewage and textile industry wastewaters	15min	0.5 9	Turbidity	125 to 6NTU	99, 100
					Turbidity	58 to 14NTU and 97 to 80NTU	
					SS	335 to 55ppm and 295 to 50ppm	
					TDS	265 to 205ppm and 345 to 295ppm	
Hydrolysed polyacrylamide-grafted-Tamarind kernel polysaccharide (Hyd. TKP-g-PAM)	Microwave-assisted	Kaolin suspension Municipal sewage wastewater	15min	0.5 9	Turbidity	125 to 6NTU	92
					Turbidity	58 to 6NTU	
					SS	335 to 20ppm	
					TDS	265 to 190ppm	
					COD	540 to 155ppm	

3.1 Grafting methods

Grafted copolymers consist of a long sequence of one polymer with one or more branches of another polymer.¹⁰¹ With the help of preformed polymer (polysaccharide in case of grafted polysaccharides) the synthesis of graft copolymer process will start. The free radical sites will be created on this preformed polymer with the help of external agent. The agent should be effective enough to create the required free radical sites, and at the same time should not be too drastic to rupture the structural integrity of the preformed polymer chain. Once the free radical sites are formed on the polymer backbone, the monomer can be added up through the chain propagation step, leading to the formation of grafted chains.^{85, 102} Conventional redox, microwave-initiated and microwave-assisted grafting methods have been reported to have successfully synthesised plant-based grafted bio-flocculants.^{93, 98-100} Figure 6 shows the synthesis of grafted copolymers of carboxymethyl starch (CMS), tamarind kernel polysaccharide (TKP), and sodium alginate (SAG)

with acrylamide as monomer by using conventional, microwave-initiated and microwave-assisted method.¹⁰⁰

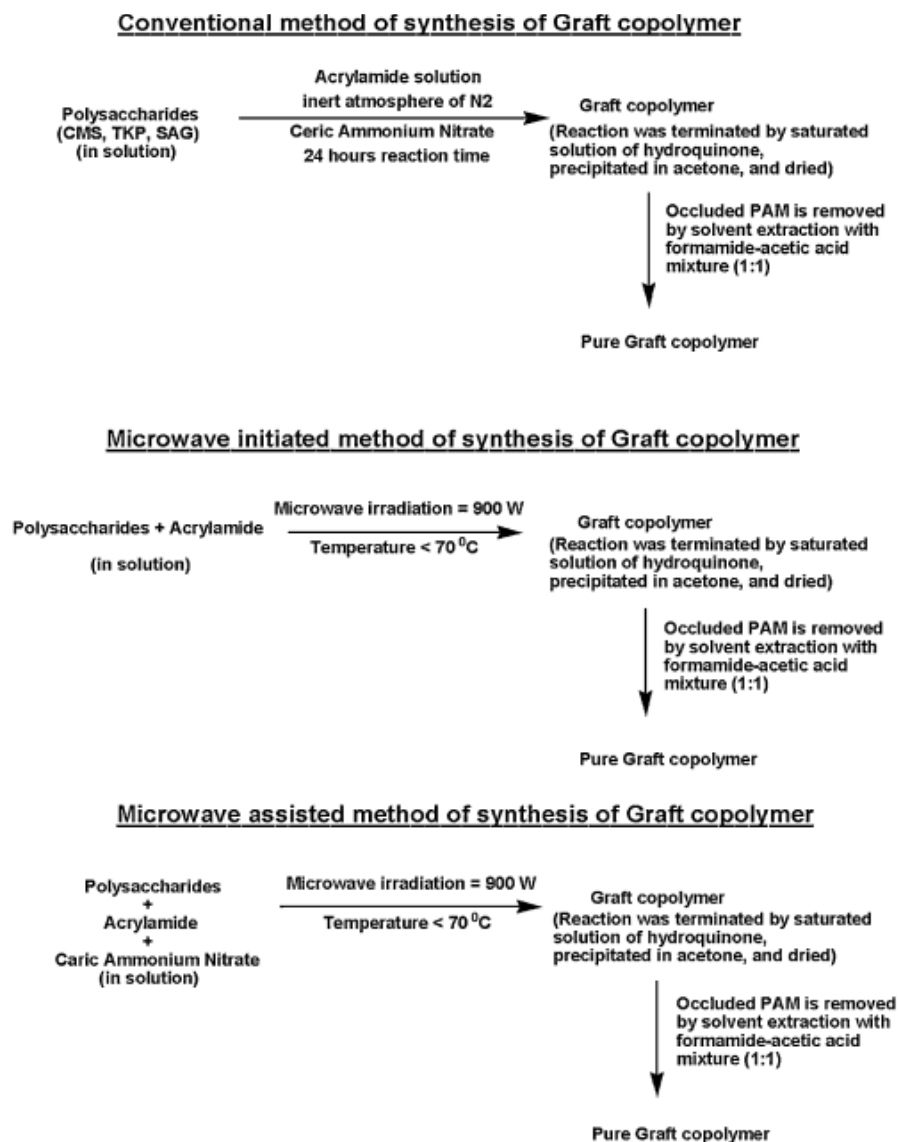


Figure 6. Schematic representation for the synthesis of grafted copolymers using conventional, microwave-initiated and microwave-assisted method¹⁰⁰

Conventional redox (or conventional free radical) grafting method using chemical free radical initiators (e.g. ceric ammonium nitrate (CAN))^{41, 88, 94-96} with nitrogen as the inert gas is commonly used to synthesise grafted copolymers. However, conventional grafting method has the drawback of undesired homopolymer formation in the concurrent competing reaction. This would decrease the copolymer yield, contaminate the copolymeric product and cause problem in the commercialisation of the grafting procedures.⁸⁴ Normally, the process requires prolonged Soxhlet extraction using a mixture of formamide and acetic acid to remove all the homopolymer

and/or unreacted substrates from the copolymer surface.^{100, 103} Moreover, the requirement of an inert atmosphere is an added disadvantage.

Recently, the synthesis of grafted copolymers used microwave based techniques to alleviate the limitations in the synthesis of a range of grafted modified polysaccharide materials.^{25, 84} It may be used along with a chemical free radical initiator (microwave assisted technique)^{90, 104} or even without any chemical free radical initiator (microwave initiated technique).¹⁰⁵ Microwave based techniques have certain advantages over other conventionally used techniques for free radical generation. It is reliable, easy to operate and highly reproducible. Hence, they offer a promising breakthrough for synthesis of grafted copolymers and encourage their further utilisation in various applications.^{84, 85} As listed in Table 3, plant-based grafted bio-flocculants have been successfully synthesised with microwave-assisted and microwave-initiated techniques by using acrylamide or methacrylic acid as the monomers.^{92, 93, 97, 99, 100} Grafted bio-flocculants synthesised by microwave initiated and microwave assisted methods were proved to provide a better quality grafted copolymers with higher percentage of grafting in comparison with conventional redox grafting method.^{99, 100} Some research findings showed that the grafted copolymers synthesised by microwave-assisted method presented superior flocculation characteristics when compared with grafted flocculants synthesised by conventional and microwave initiated methods as well as with commercially available non-ionic flocculant (Rishfloc 226 LV).^{99, 100}

Even though grafted bio-flocculants synthesised with microwave technology present superior flocculating characteristics but its usage is concerned with high production cost. In the earlier studies, most of the grafting processes are carried out using domestic microwave ovens in which the irradiation power is generally controlled by on/off cycles of the magnetron.⁸⁴ Their use is not encouraged due to the safety concerns caused by insufficient control over the reaction temperature and pressure. To overcome these issues, modifications to domestic microwave ovens have been made but it is associated with high equipment cost which would increase the production cost of the copolymers and severely limit the application of grafted bio-flocculants in developing countries. For microwave based methods, proper care has to be taken to keep the reaction mixture below the boiling point. This is done to minimise the competing homopolymer formation and also to prevent formation of unwanted vapours, which may be toxic/carcinogenic due to presence of acrylamide.⁹³

3.2 Flocculation efficiency

As shown in Table 4, plant-based grafted bio-flocculants have been successfully synthesised and applied in treatment of various types of wastewaters or effluents, and resulted in higher decrease in the environmental-concerned parameters such as solids, turbidity, dyes and COD. Commonly, polyacrylamide is chosen for synthesis of grafted copolymers. Recent studies have shown that other types of chemical polymers such as polyacrylonitrile,⁹⁶ polymethacrylic acid,⁹⁷ polymethylmethacrylate⁹⁸ were successfully used in production of grafted bio-flocculants with high removal capability in solids and turbidity.

As expected, the grafted bio-flocculants have shown better flocculation efficacy than the natural polymers (ungrafted). This is due to the higher hydrodynamic volume (i.e. intrinsic viscosity) of the former⁹³ which leads to higher flocculation efficacy.¹⁰⁶ For example, *Plantago psyllium* mucilage grafted polyacrylamide (Psy-g-PAM) copolymer was proved to be a better flocculant than pure Psyllium mucilage in the treatment of tannery and domestic wastewater.⁹⁴ In another study, polyacrylamide grafted *Tamarindus indica* mucilage (Tam-g-PAM) showed better flocculation efficiency than the pure mucilage for removal of various types of dyes from model textile wastewater containing azo, basic, and reactive dyes.⁴¹ Another study reported that polyacrylamide grafted tamarind kernel polysaccharide (TKP-g-PAM) synthesised by microwave-assisted grafting method was superior to TKP and polyacrylamide-based commercial flocculant (Rishfloc 226 LV) in flocculation tests.⁹⁹

In a study, hydrolysed polyacrylamide-grafted tamarind kernel polysaccharide (Hyd. TKP-g-PAM)⁹² was synthesised and shown to surpass the flocculation characteristics of unhydrolysed grafted copolymer (TKP-g-PAM) in treatment of kaolin suspension and municipal sewage wastewater. It was reported that after hydrolysis, the grafted chains become more straightened and expanded but still have flexibility compared with the unhydrolysed chains.¹⁰⁷ These properties result in a higher radius of gyration as well as pervaded volume and hence a higher flocculation efficiency obtained.¹⁰⁶

The flocculating efficiency of grafted bio-flocculants obtained with different grafting methods is compared in some studies. In a research work, the flocculation performance of the optimised grade of grafted copolymers (TKP-g-PAM) synthesised by microwave assisted method showed the best flocculation efficiency followed by optimised grafted copolymers synthesised by microwave initiated method and lastly free radical initiated grafting method in the treatment of synthetic wastewater (kaolin suspensions) and municipal sewage wastewater.¹⁰⁰ Another study⁹⁹ also shows that microwave synthesis process produced longer grafted copolymers (TKP-g-PAM) with higher molecular weight and displayed better flocculating efficiency compared to the grafted bio-flocculant synthesised with conventional method.

As a concluding remark, grafted copolymers synthesised by microwave initiated and assisted method display better flocculation efficiency with higher percentage of grafting compared to conventional redox grafting method.¹⁰⁰ The higher the percentage of grafting, the longer the grafted chains with higher molecular weight and radius of gyration could be obtained. With increase in molecular weight and radius of gyration, the approachability of the contaminants towards the grafted copolymers will be increased and as the results better flocculation efficiency could be achieved.^{106, 108}

3.3 Flocculation mechanism

For grafted bio-flocculants, it was reported that the increased in flocculation efficiency was contributed by increased in chain length and molecular weight.⁹⁴ Polyacrylamide grafted polysaccharides are mainly non-ionic or anionic in nature⁹² while the sustained organic and inorganic matters in wastewater carry low negative charges or positive charges. The possible

reason for better flocculation characteristics of graft copolymers over the ungrafted bio-flocculant is essentially because of polymer bridging mechanism, because the segments of a polymer chain can adsorb onto different particles surface and form bridges between adjacent particles. As the grafted bio-flocculants exhibit the characteristics of long polymer chains (high molecular weight) and high radius of gyration, hence the adsorbed polymer molecules tend to adopt more extended configuration for interacting with more than one particle and lead to effective formation of particle-polymer-particle complex (flocs).^{87, 109}

As reported in a study,⁹⁹ polymer bridging was responsible for better flocculating property of the grafted copolymers over linear polymer where the segments of a polymer chain were adsorbed onto different particles, thus linking the particles together. Some studies^{94, 95} show that there was an optimal dosage at which the flocculation efficacy was maximum (i.e. the turbidity of the supernatant collected was minimum). Beyond the optimum dosage, the excess grafted bio-flocculant would cause the aggregated particles (flocs) to redisperse in the suspension and would also reduce particle settling and finally decrease the flocculation (i.e. turbidity of the collected supernatant increases). This behaviour of the flocculation curve was reported to be corresponded with the bridging mechanism involved behind the phenomenon.^{88, 93} Another study stated that due to the flexible polyacrylamide graft chains, the colloidal particles aggregate through bridging effect and form larger net-like flocs. Then, with the help of enhanced approachability of polyacrylamide chains, the larger flocs with net-like structure can further seize residual particles from water through sweeping effect.¹¹⁰ Finally, the compacted flocs are formed and settle down.

Most of the reported studies in this field are focused mainly in application of grafted bio-flocculants in wastewater treatment. The investigation of its flocculation mechanism is very limited. Since the chain length and molecular weight are the key factors to determine the main flocculation mechanism underlying the flocculating performance of grafted bio-flocculants, it is proposed that measurement of molecular weight and observation of flocs formation with dynamic light scattering techniques for ungrafted and grafted bio-flocculants could be applied to identify the responsible flocculation mechanism.

3.4 Current challenges and future direction

The on-going research on plant-based grafted bio-flocculants is conducted on laboratory scale in treatment of wastewaters or effluents. There are many factors that constraint its development and application to pilot scale or in other industries.

The monomer consumption and the chemicals used in the synthesis process may have the impact on the environment and the application of these grafted bio-flocculants in food or pharmaceutical industries may cause health and safety issues. A study indicated that acrylamide is neurotoxic in animals and humans, and it has been shown to be a reproductive toxicant in animal models and a rodent carcinogen.¹¹¹ For acrylonitrile, studies showed that it is a mutagen, a tetragen and a carcinogen.¹⁰³ This factor would limit its application in those industries that produce human consumed products.

Another problem is the complexity of the synthesis process. To synthesise grafted copolymers, the plant-derived bio-flocculants is produced first and followed by grafting of monomers onto the backbone of polysaccharides. The whole production process is much more time and energy consuming compared to production of plant-based bio-flocculants. In addition, even though it was stated in the literature that the grafted bio-flocculants are biodegradable to the desirable extent but no study has conducted to prove it and its biodegradability is still unknown.

Another possible difficulty with the commercialisation of grafted bio-flocculants could be the scale up. Higher energy input is required for larger quantities and relatively high equipment cost is needed if microwave grafting method is going to be employed for the synthesis. Furthermore, there is a lack of investigations on applying grafted flocculants in treatment of wastewaters on a consistent basis.

In order to address all the above mentioned challenges, comprehensive investigation is required to prove their validity and promote their industrial applications. More research is needed to derive maximum benefits of microwave grafting technology and grafted bio-flocculants in order to balance the high cost of scaling up and operation. Optimisation of the grafting conditions is essential to ensure the continuous reproducibility and cost-effectiveness which are the prerequisite to meet practical quality. Furthermore, the effect of the flocculants to the environment and human should be determined and appropriate approvals obtained before it is used in water and wastewater treatment in large scale.

4. Conclusions

Due to the increasing demand of environmental friendly technologies in industry, utilisation of natural flocculants for turbidity and contaminants removal represents an important progress in sustainable environmental technology. They are nontoxic, biodegradable, can be obtained from renewable resources and their application is directly related to the improvement of quality of life for underdeveloped communities. Several studies have been conducted to investigate the flocculating properties or behaviour of plant-based bio-flocculants in wastewater treatment. The results demonstrated that they are technically promising as flocculants with high removal efficiency of solids, turbidity, colour and dye. However, its development is constrained with variation of flocculating efficiency, fast degradation, and high production cost.

Modification of natural polymers with chemical grafting has been studied recently to improve the flocculating characteristics. The developed plant-based grafted bio-flocculants essentially combine the best properties of both natural and synthetic polymers and exhibit higher flocculating performance. However, the complexity of the synthesis process, the environmental issue and safety concern of the grafting process, the extent of biodegradability and high production cost are the current challenges to be overcome. Bridging was reported to be the responsible mechanism underlying the flocculating action of bio-flocculants.

All the studies about plant-based bio-flocculants took place under lab-scale conditions and their applications in industry are still at their infancy. Therefore, possible applications of natural flocculants in different industries including food and beverage, mineral, papermaking and oleo-

chemical are suggested in this study to highlight the potential utilisation of bio-flocculants in diverse sectors. For the sake of ecology and human health, more qualitative and quantitative research are necessary to be carried out to further exploit the applications of plant-derived bio-flocculants in different industries and to address all the issues mentioned above.

Associated Content

Supporting Information Available: Background of processing industries involving clarification/flocculation process. This material is available free of charge via the internet at <http://pubs.acs.org>.

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