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A comprehensive review on harvesting of microalgae using Polyacrylamide-Based Flocculants: Potentials and challenges

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ABSTRACT

Microalgae biomass is touted as a highly promising source of renewable third-generation biofuels that could enable a lucrative transition from conventional fossil fuels to more sustainable and environment-friendly energy alternatives. A significant limiting step for large-scale microalgae production and utilization is harvesting and dewatering the cultivated biomass, which comprise 20–30% of the total production expenses. Compared to traditional physical harvesting methods, coagulation-flocculation techniques using polyacrylamide-based flocculants have garnered attention as promising alternatives due to their high harvesting efficiencies, cost-effectiveness, convenience, and scalability. This paper delivers an up-to-date progress in the harvesting of microalgae suspensions using various polyacrylamide flocculants. For the first time, a comprehensive evaluation of existing harvesting studies for freshwater and marine microalgae species using polyacrylamide-based flocculants was conducted. The impact of polyacrylamide-based flocculant characteristics (e.g., charge type, charge density, polymer architecture, molecular weight) on flocculation efficiencies was examined. The effect of the culture medium properties (e.g., pH, salinity, microalgae species, microalgae growth phase, cell density, flocculation aids) on polyacrylamide-induced flocculation was also evaluated. Existing pilot-scale and large-scale polyacrylamide-based flocculation studies were explored. The review further identifies the research gaps, key challenges and future prospects for optimizing microalgae flocculation studies.

1. Introduction

Harvesting microalgae biomass has been gaining prominence in recent years due to their versatility for a wide range of industrial applications. Microalgae have been noted to efficiently utilize energy from sunlight to generate a vast range of valuable products, even exceeding plant crops [1,2]. These products include pigments (e.g. chlorophylls, carotenoids), lipids, carbohydrates, proteins, nucleic acids, vitamins, antioxidants and nutraceuticals, all of which are obtained from the same harvested batch of microalgae biomass [3,4] . These high-value biomass compounds can be directed to biofuel production as well as pharmaceutical applications. These primary constituents derived from the biomass are transformed to various products via chemical, enzymatic and microbial deconstruction [5].

Recently, microalgae biomass has been increasingly exploited to derive various forms of biofuels such as biodiesel, biohydrogen, bio-oil, biogas and bioethanol that are touted as potential alternatives to conventional fossil fuels [6–8]. Despite extensive research endeavors, economic feasibility has not yet been achieved for commercial application of most microalgae harvesting technologies for biofuel production. The major limitations that inhibit microalgae biomass production and utilization for biofuel generation on a large scale are the energy-intensive

Acronyms: PAM, Polyacrylamide; CD, Charge density; MW, Molecular weight; St-g-PAM, Polyacrylamide-grafted starch; PADB, Poly(acrylamide-acryloyloxyethyl trimethyl ammonium chloride-butyl acrylate); SEM, Scanning electron microscopy; AOM, Algogenic organic matter; EPS, Extracellular polymeric substances; DADMAC, Polydiallyldimethylammonium chloride; PUFA, Polyunsaturated fatty acids; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; LC_{50}, Lethal Concentration 50.

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processes involved and the correspondingly high operational expenses. Among these, harvesting and dewatering techniques for microalgae cultures pose significant technological barriers in scaling up biofuel generation from microalgae biomass. This is mainly due to the small sizes and densities of microalgae cells (typically in the range 2–50 μm) encountered that remain suspended in their cultures, as well as the dilute concentrations of microalgae cells present in their cultures (typical concentrations of 0.5–5 g/L) [9].

The main processes implemented in the generation of biofuels from microalgae biomass are microalgae cultivation, biomass harvesting, dewatering, disrupting microalgae cells, lipids extraction, and transformation of microalgae lipids into biodiesel [10,11]. The costs associated with the harvesting step alone can contribute to 20–30% of the total production costs, depending on the type of method applied. Hence, harvesting microalgae biomass has been acknowledged as a cost-determining step in biofuel production [12]. Harvesting techniques serve a dual purpose of concentrating microalgae biomass in dilute cultures for biofuel production and reclaim large quantities of water for reuse. The concentration of microalgae biomass is generally performed in two stages. The primary stage involves concentrating the dilute cultures up to 2–7% TSS. This is followed by a secondary stage where the microalgae slurry is dewatered resulting up to 15–25% TSS. The concentration of cells can be determined through optical density, chlorophyll content, dry weight and ash-free dry weight measurements [13].

Microalgae biomass is traditionally harvested by a wide range of physical, chemical, and biological processes. These techniques can be employed on their own or in combination with other techniques to optimize the harvesting process. Commercialization of biofuels derived from microalgae entails the application of one or more of these harvesting techniques in an efficient, sustainable and cost-effective manner. Physical harvesting techniques typically encompass gravity sedimentation, filtration, centrifugation, flotation, electro-coagulation and magnetic flocculation processes [14–17]. These techniques demonstrate high recoveries for harvesting microalgae biomass and mostly offer uncontaminated biomass suitable for extracting high-value products. On the other hand, the techniques are marred by high associated operational costs, high energy demand and prolonged durations for harvesting [16,18–20].

Chemical harvesting techniques essentially include coagulation-flocculation processes where chemical additives are introduced into microalgae suspensions to induce aggregation. These processes have been established as the most suitable harvesting techniques in terms of energy demand, operational costs, potential environment-friendly approach and high efficiencies in concentrating microalgae biomass from their cultures [21–25]. The availability of a large variety of flocculants and the simple, standard operation associated with the methods render chemical harvesting techniques as highly reliable and scalable for large-scale applications. Furthermore, biological harvesting techniques incorporate auto-flocculation at high medium pH, microbe-assisted flocculation or bio-flocculation instigated by extracellular polymeric substances secreted by microalgae cells [15,26–29]. They are characterized by low energy demand, absence of any toxic chemical additives and lack of interference with the lipid extraction process from the harvested biomass. Associated drawbacks of the methods include biomass contamination with microbes, requirement of nutrients and specific growth conditions [2,7,18,21].

Polyelectrolyte flocculants can be employed to flocculate dilute microalgae cultures up to a concentration factor of 800, facilitating dewatering operations for the microalgae biomass. The biomass can be concentrated further by 10-fold via treatment with a low-power centrifuge or filtration following the flocculation process [30]. Polyacrylamides are the most commonly employed synthetic organic polyelectrolytes for harvesting microalgae. Polyacrylamides of various charge types have been extensively applied for treating drinking water and clarifying wastewaters from municipal and industrial processes. Commercial high-molecular-weight polyacrylamides are typically stable, non-toxic, readily available and cost-effective, and offer excellent harvesting efficiencies when applied to microalgae suspensions at low flocculant doses [31].

For the first time, an exhaustive review was accomplished to summarize all flocculation studies for freshwater and marine microalgae species using polyacrylamide-based flocculants in existing literature. The effect of polyacrylamide-based flocculant characteristics (such as charge type, charge density, polymer architecture and molecular weight) on harvesting efficiencies was elucidated. The impact of the culture properties (such as pH, salinity, microalgae species, microalgae growth phase, cell density, flocculation aids) on polyacrylamide-induced flocculation was also assessed. Microalgae harvesting studies using polyacrylamides conducted in pilot-plant and large scale were also reported in this paper. Further, the main challenges encountered with the flocculation processes and the application of polyacrylamides for harvesting microalgae were explored, and associated research gaps were addressed.

2. Evaluation of Polyacrylamide-Based flocculants applied in microalgae harvesting studies

2.1. Summary Table for Polyacrylamide-Based flocculants utilized in microalgae harvesting studies

Polyacrylamide polymers are one of the most extensively utilized flocculants in various water treatment applications including wastewater treatment and microalgae harvesting. Numerous studies have been conducted to evaluate the efficiencies of several polyacrylamides in harvesting different microalgae species via flocculation under varying flocculation conditions, which will be discussed in this section (summarized in Table 1). These studies successfully elucidate the influence of structural and functional group characteristics of polymers, including their charge densities and molecular weights on their flocculation behavior in microalgae suspensions. Other factors regulating the flocculation efficiencies include flocculant dose, microalgae strains, biomass concentration, suspension pH and ionic strength. Advances in polymer modification techniques have enabled the synthesis of efficient polymeric flocculant designs considering these factors to effectively target and flocculate microalgae from their stable suspensions through specific physical and chemical forces of interaction.

2.2. Polyacrylamide flocculation efficiency against inorganic coagulants and natural flocculants

Several flocculation studies have the illustrated superior flocculation behavior of polyacrylamides over conventional inorganic flocculants and natural flocculants. For instance, flocculation studies on *Chlorella vulgaris* microalgal suspensions were performed by Vu et al. [42] by individually employing three kinds of flocculants - inorganic salts of ferric chloride and aluminum sulfate, a synthetic organic cationic polyacrylamide FLOPAM FO 4808 and natural organic polymer chitosan. Comparative analyses between the flocculants revealed that the synthetic polyacrylamide (possessing a high molecular weight and high charge density) was the most efficient flocculant for harvesting microalgae. The flocculant rendered significant reductions in optical density reaching 96% at polymer doses as low as 20 mg/L microalgae suspension.

Further, raising polyacrylamide doses beyond 100 mg/L proved counterproductive as optical densities began to increase. This phenomenon reflects the underlying mechanisms of flocculation with polyacrylamide as the cationic flocculant neutralizes the surface charges of microalgae followed by bridging microalgae cells. An overdose of the flocculant results in complete surface coverage and subsequent electrostatic repulsions, thereby restabilizing microalgal suspensions.

In comparison, inorganic salts of ferric chloride and aluminum sulfate only managed to decrease the optical density up to 86% and 77% for

Table 1
Summary Table for Existing Literature Studies Based on The Application of PAM-Based Flocculants Microalgae Harvesting.

PAM Name	PAM Properties	PAM Type	Microalgae Species	Marine / Freshwater	Flocculation conditions	Flocculation Efficiency (%)	Refer-ences
Magnafloc LT 225	–	Cationic	• *Chlorella* sp. • *Chlamydomonas reinhardtii* • *Scenedesmus acuminatus*	Freshwater	• Cell Concentration (cells/mL): 10^7 • Dose (mg/L): 35 35 30 • Settling time (min): 30 • pH: 7	72%95%95%	[32]
POLY SEPAR PK 55H	High CDHigh MW	Cationic	• *Chlorella* sp. • *Chlamydomonas reinhardtii* • *Scenedesmus acuminatus*	Freshwater	• Cell Concentration (cells/mL): 10^7 • Dose (mg/L): 1.5 4 2 • Settling time (min): 30 • pH: 7	95% 99% 98%	[32]
POLY SEPAR KW 745H	–	Cationic	• *Chlorella* sp. • *Chlamydomonas reinhardtii* • *Scenedesmus acuminatus*	Freshwater	• Cell Concentration (cells/mL): 10^7 • Dose (mg/L): 20 20 20 • Settling time (min): 30 • pH: 7	89% 90% 89%	[32]
Magnafloc LT 27	High CD Very High MW	Anionic	• *Chlorella* sp. • *Chlamydomonas reinhardtii* • *Scenedesmus acuminatus*	Freshwater	• Cell Concentration (cells/mL): 10^7 • Dose (mg/L): 10 40 10 • Settling time (min): 30 • pH: 7	8% 21% 8%	[32]
Magnafloc LT 25	Medium CD Very High MW	Anionic	• *Chlorella* sp. • *Chlamydomonas reinhardtii* • *Scenedesmus acuminatus*	Freshwater	• Cell Concentration (cells/mL): 10^7 • Dose (mg/L): 10 40 10 • Settling time (min): 30 • pH: 7	5% 24% 4%	[32]
POLY SEPAR AN 10 TW	–	Anionic	• *Chlorella* sp. • *Chlamydomonas reinhardtii* • *Scenedesmus acuminatus*	Freshwater	• Cell Concentration (cells/mL): 10^7 • Dose (mg/L): 20 20 20 • Settling time (min): 30 • pH: 7	10% 20% 10%	[32]
Magnafloc LT 20	Medium MW	Non-ionic	• *Chlorella* sp. • *Chlamydomonas reinhardtii* • *Scenedesmus acuminatus*	Freshwater	• Cell Concentration (cells/mL): 10^7 • Dose (mg/L): 10 10 20 • Settling time (min): 30 • pH: 7	3% 12% 10%	[32]
POLY SEPAR AN 20	–	Non-ionic	• *Chlorella* sp. • *Chlamydomonas reinhardtii* • *Scenedesmus acuminatus*	Freshwater	• Cell Concentration (cells/mL): 10^7 • Dose (mg/L): 10 10 20	5% 3% 1%	[32]

(*continued on next page*)

Table 1 (*continued*)

PAM Name	PAM Properties	PAM Type	Microalgae Species	Marine / Freshwater	Flocculation conditions	Flocculation Efficiency (%)	Refer-ences
					• Settling time (min): 30 • pH: 7		
Zetag 8819	High CD High MW	Cationic	• Chlorella sp.	Freshwater	• Cell Concentration (mg/L): 720 • Settling time (min): 60 • Dose (mg/L): 34	98%	[33]
Magnafloc E-38	High MW	Anionic	• Chlorella zofingiensis	Freshwater	• Cell Concentration (mg/L): 720 • Settling time (min): 60	≈ 0%	[33]
Zetag 7570	Medium CD Medium MW	Cationic	• Nannochloropsis salina	Marine	• Cell Concentration (g/L): 0.29–0.40 • Settling time (min): 5 • Dose (mg/L): 3 • pH: 8.5–9	92.4%	[34]
Zetag 8190	Very High CD High MW	Cationic	• Tetraselmis suecica	Marine	• Cell Concentration (g/L): 0.29–0.40 • Settling time (min): 5 30 • Dose (mg/L): 10 8	 95% 97%	[34]
Zetag 8180	High CD High MW	Cationic	• Tetraselmis suecica	Marine	• Cell Concentration (g/L): 0.29–0.40 • Settling time (min): 5 30 • Dose (mg/L): 10 8	 14% 92.5%	[34]
Zetag 8140	Medium CD High MW	Cationic	• Tetraselmis suecica	Marine	• Cell Concentration (g/L): 0.29–0.40 • Settling time (min): 5 30 • Dose (mg/L): 8 4	 15% 55%	[34]
Zetag 7557	High CD High MW	Cationic	• Phaeodactylum tricornutum • Neochloris oleoabundans	Marine	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 10 • Settling time (min): 120 • pH: 7	98% 52%	[35]
Synthofloc 5080H	High CD High MW	Cationic	• Phaeodactylum tricornutum • Neochloris oleoabundans	Marine	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 10 • Settling time (min): 120 • pH: 7	93% 36%	
Magnafloc 351	High MW	Non-ionic	• Phaeodactylum tricornutum • Neochloris oleoabundans	Marine	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 10 • Settling time (min): 60 • pH: 7	0% 0%	[35]
Synthofloc 5080H	High CD High MW	Cationic	• Neochloris oleoabundans	Marine	• Cell Concentration (g/L): 0.8 • Dose (mg/L): 30 • pH: 7 • Salinity (g/L): 35	97%	[36]
Synthofloc 5040H	Medium CD High MW	Cationic	• Neochloris oleoabundans	Marine	• Cell Concentration (g/L): 0.8 • Dose (mg/L): 30 • pH: 7 • Salinity (g/L): 35	93%	[36]
Synthofloc 5025H	Low CD High MW	Cationic	• Neochloris oleoabundans	Marine	• Cell Concentration (g/L): 0.8 • Dose (mg/L): 30	88%	[36]

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Table 1 (*continued*)

PAM Name	PAM Properties	PAM Type	Microalgae Species	Marine / Freshwater	Flocculation conditions	Flocculation Efficiency (%)	Refer-ences
					• pH: 7 • Salinity (g/L): 35		
Flopam FO 4990	High CD (100%) High MW (4.5–7.1 MDa)	Cationic	• Nannochloropsis salina	Marine	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 20 • Settling Time (min): 60	93%	[37]
Flopam FO 4800	High CD (80%) High MW (4.9–7.1 MDa)	Cationic	• Nannochloropsis salina	Marine	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 20 • Settling Time (min): 60	83%	[37]
Flopam FO 4650	Medium CD (55%) High MW (4.5–7.1 MDa)	Cationic	• Nannochloropsis salina	Marine	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 20 • Settling Time (min): 60	80%	[37]
Flopam FO 4550	Low CD (45%) High MW (4.1–7.1 MDa)	Cationic	• Nannochloropsis salina	Marine	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 20 • Settling Time (min): 60	73%	[37]
Flopam FO 4800	High CD (80%) High MW (4.9–7.1 MDa) Linear	Cationic	• Chlamydomonas reinhardtii	Freshwater	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 19	97%	[38]
Flopam FO 3801	High CD (80%) High MW (4.9–7.1 MDa) Branched	Cationic	• Chlamydomonas reinhardtii	Freshwater	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 30	98%	[38]
Flopam FO 4990	High CD (100%) High MW (4.5–7.1 MDa)	Cationic	• Chlamydomonas reinhardtii	Freshwater	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 28	96%	[38]
Flopam FO 4280	Low CD (15%) High MW	Cationic	• Chlamydomonas reinhardtii	Freshwater	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 60	97%	[38]
Flopam AN 910 (coupled with polyamine)	Low CD (10%) High MW	Anionic	• Chlamydomonas reinhardtii	Freshwater	• Cell Concentration (g/L): 0.7 • Dose (mg/L): 40	20%	[38]
71,301 (Nalco)	Medium CD Medium/High MW	Cationic	• Chlorococcum sp.	Marine	• Cell Concentration (g/L): 0.6 • Dose (mg/L): 3 • Settling Time (min): 30	78%	[39]
71,303 (Nalco)	Low/Medium CD Medium MW	Cationic	• Chlorococcum sp	Marine	• Cell Concentration (g/L): 0.6 • Dose (mg/L): 4 • Settling Time (min): 30	90%	[39]
71,305 (Nalco)	Low CD Medium/High MW	Cationic	• Chlorococcum sp	Marine	• Cell Concentration (g/L): 0.6 • Dose (mg/L): 3 • Settling Time (min): 30	85.3%	[39]
82,230 (Nalco)	Low/Medium CD Medium/High MW	Anionic	• Chlorococcum sp.	Marine	• Cell Concentration (g/L): 0.6 • Dose (mg/L): 5 • Settling Time (min): 30	84.5%	[39]
Magnafloc 155	Low/Medium CD High MW	Anionic	• Chlorococcum sp.	Marine	• Cell Concentration (g/L): 0.6 Dose (mg/L): 2 • Settling Time (min): 30	83.9%	[39]
Magnafloc 156	Medium CDHigh MW	Anionic	• Chlorococcum sp.	Marine	• Cell Concentration (g/L): 0.6 • Dose (mg/L): 3 • Settling Time (min): 30	84.5%	[39]
Magnafloc 351	High MW	Non-ionic	• Chlorococcum sp.	Marine	• Cell Concentration (g/L): 0.6 • Dose (mg/L): 10 • Settling Time (min): 30	80%	[39]
Flopam FO 3801	High CD High MW	Cationic	• Synechocystis sp. • Chlorella vulgaris • Phaeodactylum tricornutum	• Freshwater • Freshwater • Marine	**Lab Scale** • Cell Concentration (g/L): 0.160 0.232 0.168 • Dose (mg/g):	98.9% 92.3% 90%	[40]

(*continued on next page*)

Table 1 (*continued*)

PAM Name	PAM Properties	PAM Type	Microalgae Species	Marine / Freshwater	Flocculation conditions	Flocculation Efficiency (%)	Refer-ences
					31.25 34.5 35.7		
Flopam FO 3801	High CD High MW	Cationic	• Synechocystis sp. • Chlorella vulgaris • Phaeodactylum tricornutum	• Freshwater • Freshwater • Marine	• Growth phase: Stationary **Pilot scale** • Dose (mg/g): 44.643.135.7 • Settling time: 10 min24 hrs	10 min 87.5% 82.7% 87.2% 24 hrs 98.6% 75.2% 90.3%	[40]
Flopam FO 4808	High CD High MW	Cationic	• Synechocystis sp. • Chlorella vulgaris • Phaeodactylum tricornutum	• Freshwater • Freshwater • Marine	• Cell Concentration (g/L): 0.160 0.232 0.168 • Dose (mg/g): 31.3 25.9 23.8 • Growth phase: Stationary	98.5% 98.4% 90%	[40]
Percol PR 8400	Medium CD High MW	Cationic	• Chlorella sp.	Freshwater	• Cell Concentration (g/L): 0.38 • Dose (mg/L): 10 • pH: 6.5	94%	[41]
Flopam FO 4808	High CD High MW	Cationic	• Chlorella vulgaris	Freshwater	• Cell Concentration (g/L): 0.36 • Dose (mg/L): 20 • Settling Time (hr): 1	96%	[42]
Zetag 3815	High CD (>80%) High MW (>15 MDa)	Cationic	• Chlorella vulgaris	Freshwater	• Cell Concentration (g/L): 0.7 • Dose (mg/g): 36 • Settling Time (min): 1	80 ± 4.5	[43]
Flopam FO 4808	High CD (>80%) High MW (>15 MDa)	Cationic	• Chlorella vulgaris	Freshwater	• Cell Concentration (g/L): 0.7 • Dose (mg/g): 36 • Settling Time (min): 1	95 ± 5.0%	[43]
Flopam FO 3801	High CD (>80%) High MW (>15 MDa)	Cationic	• Chlorella vulgaris • Phaeodactylum tricornutum	FreshwaterMarine	• Cell Concentration (g/L): 0.370 0.508 • Dose (mg/g): 18.9 13.7 • Settling Time (min): 1 • pH − 4–10	90% 99%	[44]
Commercial PAM coupled with Bentonite	(CPAM, DS = 0.5, Mw = 8 × 10^6 Da)	Cationic	• *Chlorella vulgaris*	Saltwater	• Cell Concentration (g/L): 1.06 • Dose (mg/L): CPAM − 1Bentonite − 80 • Settling Time (min): 3 • pH − 4–10	94%	[45]
Crystalfloc B490H (90% active ingredient powder)	High MW	Cationic	• *Microalgae colonies (Desmodesmus* sp., *Dictyosphaerium* sp., *Chlorella* sp.*)*	Freshwater	**Lab scale** • PAM Dose (mg/L): 4 • Settling time (min): 15 **Pilot scale** • PAM Dose (mg/L): 4	70%	[46]

(*continued on next page*)

Table 1 (*continued*)

PAM Name	PAM Properties	PAM Type	Microalgae Species	Marine / Freshwater	Flocculation conditions	Flocculation Efficiency (%)	Refer-ences
						40% (3 days) 63% (14 days)	
Commercial PAM	High MW	Cationic	• Scenedesmus sp. (ScS) • Scenedesmus obliquus (ScO)	Freshwater	• Cell Concentration (g/L): ScS – 2.2ScO – 2.22 • PAM Dose (mg/L): ScS – 28 ScO – 100	ScS - > 97% ScO - ≈ 99%	[23]
FLOPAM	**MW CD** ***(MDa) (mol%)***	Cationic	• *Chlorella vulgaris*	Freshwater	• Cell Density (mg/L): 260 • pH: 8	Efficiency - PAM Dose	[47]
FO 4115 SH	5.9–7.72.5					94% – 45 mg/L	
FO 4125 SH	5.9–7.74					98% – 5 mg/L	
FO 4140 SH	5.9–7.75					99% – 15 mg/L	
FO 4190 SH	6.3–8.110					99% – 1.66 mg/L	
FO 4240 SH	6.3–8.115					99% – 1.66 mg/L	
FO 4290 SH	5.9–8.520					99% – 1.66 mg/L	
FO 4350 SH	5.5–8.525					98% – 1.66 mg/L	
FO 4400 SH	4.9–7.430					99% – 1.66 mg/L	
FO 4440 SH	4.8–7.135					99% – 1.66 mg/L	
FO 4490 SH	4.6–7.140					99% – 1.66 mg/L	
FO 4550 SH	4.1–7.145					100% – 1.66 mg/L	
FO 4650 SH	4.5–7.155					99% – 1.66 mg/L	
FO 4700 SH	4.9–7.170					99% – 1.66 mg/L	
FO 4800 SH	4.9–7.180					99% – 1.66 mg/L	
FO 4990 SH	4.9–7.1100					99% – 1.66 mg/L	
FLOPAM	**MW CD** ***(MDa) (mol%)***	Cationic	• *Nannochloropsis oculata*	Marine	• Cell Density (mg/L): 290 • pH: 8	Efficiency - PAM Dose	[47]
FO 4115 SH	5.9–7.72.5					45% – 45 mg/L	
FO 4125 SH	5.9–7.74					38% – 45 mg/L	
FO 4140 SH	5.9–7.75					36% – 45 mg/L	
FO 4190 SH	6.3–8.110					54% – 45 mg/L	
FO 4240 SH	6.3–8.115					41% – 5 mg/L	
FO 4290 SH	5.9–8.520					50% – 5 mg/L	
FO 4350 SH	5.5–8.525					56% – 5 mg/L	
FO 4400 SH	4.9–7.430					63% – 5 mg/L	
FO 4440 SH	4.8–7.135					66% – 5 mg/L	
FO 4490 SH	4.6–7.140					66% – 5 mg/L	
FO 4550 SH	4.1–7.145					72% – 5 mg/L	
FO 4650 SH	4.5–7.155					81% – 0.55 mg/L	
FO 4700 SH	4.9–7.170					88% – 0.55 mg/L	
FO 4800 SH	4.9–7.180					87%- 0.55 mg/L	
FO 4990 SH	4.9–7.1100					90% – 0.55 mg/L	
ZETAG	**MWCD** ***(MDa)(mol%)***	Cationic	• *Chlorella vulgaris*	Freshwater	• Cell Density (mg/L): 260 • pH: 8	Efficiency - PAM Dose	[47]
8125	HighLow					99% – 1.66 mg/L	
8160	HighMed–high					99% – 5 mg/L	
8180	HighHigh					99%- 5 mg/L	
7652	Very highMedium					99%- 5 mg/L	
8165	Very highMed–high					99% – 5 mg/L	
8185	Very highHigh					100% – 5 mg/L	
ZETAG	**MWCD** ***(MDa)(mol%)***	Cationic	• *Nannochloropsis oculata*	Marine	• Cell Density (mg/L): 290 • pH: 8	Efficiency - PAM Dose	[47]

(*continued on next page*)

Table 1 (*continued*)

PAM Name	PAM Properties	PAM Type	Microalgae Species	Marine / Freshwater	Flocculation conditions	Flocculation Efficiency (%)	Refer-ences
8125	HighLow					44% – 45 mg/L	
8160	HighMed–high					48% – 1.66 mg/L	
8180	HighHigh					70% – 0.55 mg/L	
7652	Very highMedium					27%- 5 mg/L	
8165	Very highMed–high					51% – 5 mg/L	
8185	Very highHigh					75% – 0.55 mg/L	
Modified Cationic PAM: *Poly (acrylamideacryloyloxyethyl trimethyl ammonium chloride-butyl acrylate)*(PADB)	**MWCD *(MDa)(mol%)***	Cationic	• Green microalgae species	Freshwater	• PAM Dose (mg/L): 3.5 • Cell Density (μg/L): 18.0–78.0 • pH: 7–8		[48]
PADB1	520					95.4%	
PADB2	540					99.7%	
SOKOFLOC	**CD (mol%)**	Cationic	• *Parachlorella kessleri (CK)* • *Chlorella sorokiniana (CS)* • *Scenedesmus obliquus (ScO)*	Freshwater	• PAM Dose (mg/L): 2–5 • Settling Time (min): 8 • Growth phase: Stationary	57GP, 61GP CK: > 90%	[31]
55GP	20		• *Scenedesmus subspicatus (ScS)*		• Cell Density (g/L)):	CS: > 90%	
57GP	30 (High MW)		• *Synechococcus nidulans (SN)*		CK5.02CS7.03ScO6.35ScS6.54SN4.80	ScO: > 95%	
57GPX	30 (Low MW)					ScS: > 95%	
61GP	55					SN: > 95%	
	High MW PAMs						
FLOPAM	**CD (mol%)**	Cationic	• *Conticribra weissflogii*	Marine	• PAM Dose (mg/L): 1 • Cell Density (cells/cm^3): 3.55 × 10^5 • Settling Time (min):		[49]
FO 4140 SH	5				• Growth phase: Lag	≈93%	
FO 4240 SH	20					95%	
FO 4490 SH	40					≈88%	
FO 4700 SH	70					≈84%	
FO 4990 SH	100					≈77%	
	High MW PAMs						
Polyacrylamide-grafted starch (St-g-PAM 2)	Modified polyacrylamide-based biopolymer Grafting percentage of 907%	Cationic	• *Chlorella* sp.	Freshwater	• PAM Dose (mg/L): 0.8 • Settling Time (min): 30 • pH: 10.5	74%	[50]
Magnafloc LT 25	High MW	Anionic	• *Chaetoceros calcitrans*	Marine	• PAM Dose (mg/L): 0.1 • Settling Time (hr): 4 • pH: 10	82%	[51]

very high flocculant doses of 160 mg/L and 180 mg/L of microalgae suspension respectively. Negatively charged microalgae cells are merely neutralized by metal salts, overcoming intercellular electrostatic repulsions and undergoing flocculation[38].

Further, a poor flocculation performance of chitosan was detected as the flocculant required dosage of 200 mg/L, twenty times greater than that of polyacrylamide to attain a reduction in optical density reaching 62%. Chitosan relies less on charge neutralization and more on bridging mechanism to flocculate microalgal cells [42].

2.3. Effect of polyacrylamide charge type on microalgae flocculation

The most crucial characteristic of polymeric flocculants considered before applying them to stable microalgae suspensions is the presence of charged functional groups and their type. Uduman et al. [39] compared the efficiencies of cationic, anionic and non-ionic polyacrylamides on the flocculation of marine microalgae *Chlorococcum* sp.. All the polyacrylamide variants satisfactorily separated the microalgae from its culture, offering removal efficiencies above 78%. Cationic polyacrylamides performed the best in terms of high flocculation efficiencies and low optimum flocculant doses. Polyacrylamide variants 71,305 and 71,303 possessing low to medium charge densities and medium to high molecular weights offered the highest flocculation efficiencies of 85.3% and 89.9% at the lowest flocculant doses 3 mg/L and 4 mg/L, respectively. This is attributed to the combined effects of charge neutralization and polymer bridging interactions of polyacrylamide chains with the microalgal cells. The higher charge density of 71,303 essentially contributed to its high efficiency when compared to 71305.

Anionic polyacrylamide variants 82,230 and Magnafloc 156, bearing low to medium charge densities and medium to high molecular weights, also performed comparably to their cationic counterparts. Both 82,230 and Magnafloc 156 presented a removal efficiency of 84.5% at optimum doses 5 mg/L and 3 mg/L, respectively. Lower optimum flocculant dose for the higher molecular weight variant Magnafloc 156 reflects the key role of bridging interactions in the flocculation mechanism of microalgae suspensions using anionic polyelectrolytes. The non-ionic polyacrylamide Magnafloc 351 produced the lowest removal efficiency of 79.9% with a high optimal flocculant dose of 10 mg/L [39].

In contrast to cationic polyelectrolytes, flocculation by anionic and non-ionic polyelectrolytes occurs fundamentally employing chemical forces instead of electrostatic forces. Adsorption of polymer chains onto microalgal cells may occur via van der Waals interactions, hydrogen bonding, or chemical bonding between the functional groups on polymer chains and the microalgal cell surfaces. The unadsorbed polymer segments further interact with adjacent microalgal cells to induce flocculation by polymer bridging mechanism. Additionally, metal cations present in the marine cultures may also bridge the polymer chains and the negatively charged microalgal cells together, thereby improving the flocculation performance [52].

The effect of polyacrylamide charge type was also studied on freshwater microalgae cultures. Bleeke et al. [32] utilized a series of commercial cationic, anionic and nonionic polyacrylamides on cultures of three freshwater green microalgae species *Chlamydomonas reinhardtii, Chlorella* sp. and *Scenedesmus acuminatus.* Among the applied cationic polyacrylamides Magnafloc LT225, POLY SEPAR PK55H and KW745 H, PK55H possessed the highest molecular weight and charge density. Consequently, the polymer offered the highest flocculation efficiencies of 95%, 99% and 98% at doses 1.5, 4 and 2 mg/L for *Chlorella* sp., *Chlamydomonas reinhardtii* and *Scenedesmus acuminatus* cultures respectively. Its efficacy for harvesting the three freshwater cultures was further attested by the rapid formation of large and dense flocs. The non-ionic polyacrylamides Magnafloc LT20 and POLY SEPAR AN20 presented poor biomass recoveries below 10% at doses exceeding 10 mg/L for all three cultures. Similarly, the anionic polyacrylamides Magnafloc LT27, LT25 and POLY SEPAR AN10TW offered harvesting efficiencies between 20 and 25% for *Chlamydomonas reinhardtii* cultures, compared to other microalgae cultures with recoveries below 10%. Therefore, both anionic and non-ionic polyacrylamides were highly unsuitable for harvesting freshwater microalgae species [32].

2.4. Effect of polyacrylamide charge density on microalgae flocculation

The impact of charge density on polyacrylamide-induced flocculation was examined by Roselet et al. [47] on marine microalgae *Nannochloropsis oculata* cultures. The flocculation efficiencies of polyacrylamides considerably increased with polymer charge density alongside significant reductions in optimum flocculant doses. A clear trend for this effect is depicted in Fig. 1. For instance, at a fixed dose of 0.55 mg/L, the flocculation efficiencies rose steeply from 8 to 90% for a series of polyacrylamide variants beginning with FO 4115 SH (lowest charge density of 2.5%) to FO 4990 SH (highest charge density of 100%), respectively. Higher polyacrylamide charge densities allow for a greater extent of adsorption and charge neutralization on microalgae cell surfaces. These long-chain polymers also adsorb onto adjacent microalgae cells and bridge between them to promote aggregation. Moreover, the addition of polyacrylamides beyond the optimum doses decreased the flocculation efficiencies. The phenomenon manifests restabilization of microalgae suspensions due to overdosing of flocculants that entirely screen the negatively charged microalgae surfaces and bring about charge reversal [53].

In general, polyacrylamide flocculants with very low charge densities below 10 mol% required very high flocculant doses above 45 mg/L to effectively flocculate the marine algae cells. Polyacrylamide variants with low charge densities below 25 mol% and medium charge densities below 45 mol% required dramatically lower flocculant doses between 1.66 mg/L to 5 mg/L to achieve harvesting efficiencies surpassing 50%. Gradual suspension restabilization was detected beyond optimum doses for each of these flocculants. Finally, for polyacrylamides variants bearing high charge densities below 70 mol% and very high charge densities exceeding 80% required flocculant doses below 0.55 mg/L to offer optimal flocculation conditions with efficiencies surpassing 75%. Restabilization effects were amplified for this group of polyacrylamides when flocculants were added beyond optimum doses [47].

The impact of flocculant charge density is also very prominent in freshwater microalgae suspensions. Mikulec et al. [31] applied high molecular weight cationic Sokofloc polyacrylamide flocculants 55GP, 57GP and 61GP with charge densities of 20 mol%, 30 mol% and 55 mol % to five freshwater microalgae species, namely *Chlorella sorokiniana, Parachlorella kessleri, Synechococcus nidulans, Scenedesmus obliquus* and *Scenedesmus subspicatus*. All the polymers, especially the highest charge density and high molecular weight variants efficiently removed microalgae cells in the lag phase at small flocculant doses ranging from 2 to 5 mg/L, with recoveries surpassing 95%.

2.5. Effect of polyacrylamide molecular weight on microalgae flocculation

The impact of polyacrylamide molecular weight was not sufficiently studied enough on the flocculation efficiency in microalgae suspensions. Since most commercial polyacrylamides studied had similar ranges of high molecular weights, no profound distinctions on flocculation performance were observed, in contrast to those imparted by charge density variations. Nevertheless, Roselet et al. [47] specifically analyzed a range of commercial cationic polyacrylamides to assess the effect. In this study, high molecular weights polyacrylamides of the FLOPAM series were utilized whose weights ranged from 4100 kDa to 8600 kDa and charge densities varied from 2.5 mol% to 100%. While the influence of increasing charge density prevailed over their flocculation efficiencies in freshwater algae *Chlorella vulgaris* and marine algae *Nannochloropsis oculate* suspensions, a systematic decrease in flocculation efficiencies were also detected with increasing molecular weights at a fixed flocculant dose of 0.55 mg/L (Fig. 2). One explanation could be the lower charge densities possessed by the largest molecular weight polymers.

Polymeric flocculants with high molecular weights possess long chains that facilitate bridging mechanisms between microalgae cells, enhancing the flocculation efficiency. However, the polyacrylamide chains were unable to attain more expanded configurations to neutralize more cell surfaces in the suspension with reducing charge densities, hampering their flocculation abilities. High salinity conditions of the marine *Nannochloropsis oculate* suspensions further induced polymer coiling and lowered the flocculation performance of the cationic polyacrylamides compared to the freshwater *Chlorella vulgaris* suspensions [47].

2.6. Effect of polyacrylamide chain architecture on microalgae flocculation

Delrue et al. [38] evaluated the effect of chain structure on the flocculation ability of polyacrylamides for harvesting *Chlamydomonas reinhardtii* microalga cultures. A comparison of two cationic polyacrylamides of high charge density (80%), namely FO 4800 (linear structure) and FO 3801 (branched structure), revealed slight variations in flocculation efficiencies arising from structural differences in the polymer chains. Even though comparable flocculation efficiencies exceeding 95% were attained in terms of optical density measurements, the branched polyacrylamide variant FO 3801 required a dose of ≈ 10 g/kg of dry biomass, nearly double the optimal dose of linear polyacrylamide FO 4800 (5 g/kg) to achieve the same efficiency. Linear polyacrylamide chains of high charge density present expanded configurations in microalgae suspensions. This exposes more cationic active sites on the chain to negatively charged microalgae surfaces for charge neutralization. Further, this configuration enables the chains to bridge between and accumulate large amounts of cells than afforded by the branched architecture. Hence, linear polyacrylamides are capable of rendering large and compact flocs that settle easily. As the extent of branching increases in polyacrylamides, the exposure of these active sites decreases, affecting the flocculation behavior of the polymer chains [54].

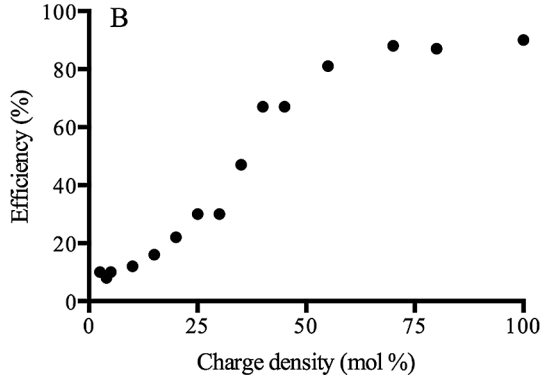

Fig. 1. Influence of polyacrylamide charge density (mol%) on the removal efficiency of Nannochloropsis oculate [47] (reused with permission from Elsevier license number 5126300827044).

2.7. Effect of different N-Substitutes on Polyacrylamide-Microalgae flocculation

Distinct flocculation performances for different N-substituted polyacrylamides were observed. Labeeuw et al. [40] reported the application of two synthetic cationic polyacrylamides FO 3801 and FO 4808 for harvesting microalgae species *Chlorella vulgaris* (eukaryotic freshwater microalgae), *Phaeodactylum tricornutum* (eukaryotic marine microalgae) and *Synechocystis* sp. (prokaryotic cyanobacterium). Both polyacrylamide variants possessed high charge densities and molecular weights but comprised of polyacrylamide backbones with different N-substitutes. Optimum flocculation performance was observed for polyacrylamides in the stationary phase of microalgal growth. Both the polymers succeeded in providing flocculation efficiencies above 90% for all three microalgae species. FO 3801 and FO 4808 polyacrylamides flocculated more than 90% microalgae cells at 23 mg/g and displayed maximum flocculation efficiencies exceeding 99% at a flocculant dose of 31.5 mg/g for *Synechocystis sp*. cultures.

Significant variations in optimum doses were noticed in eukaryotic microalgae cultures. FO 4808 was strikingly more effective for flocculating eukaryotic species than its counterpart. Flocculation efficiencies for *Chlorella vulgaris* suspensions exceeded 90% for FO 4808 at a dose of 23.5 mg/g, lower than 32 mg/g required for FO 3801. A maximum efficiency of 99% was attained for FO 4808 at a dose of 26 mg/g viz. almost half the required dose of 53 mg/g for FO 3801. Similar outcomes were obtained for *Phaeodactylum tricornutum* cultures flocculated with the two polyacrylamides. Flocculation efficiencies exceeded 90% for FO 4808 and FO 3801 at similar doses of 12 mg/g and 13 mg/g, respectively. Maximum efficiencies of 94% and 90% were achieved at doses 24 mg/g and 46.88 mg/g for FO 4808 and FO 3801, respectively, further confirming. This study successfully highlighted the role of different N-substitutes on the flocculation ability of polyacrylamides in microalgae suspensions [40].

2.8. Application of modified polyacrylamides for microalgae flocculation

A few attempts have been made to test the effectiveness of modified polyacrylamides for harvesting microalgae cells from their cultures. One

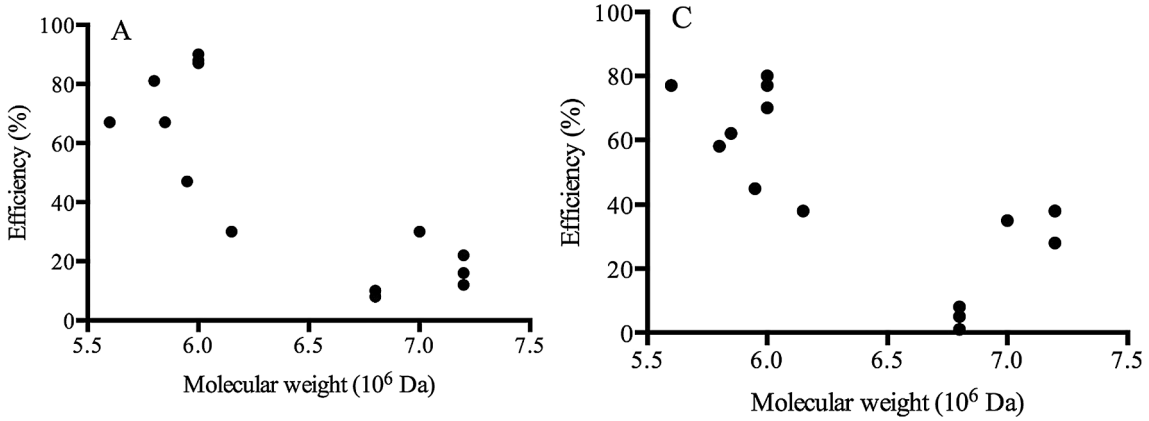

Fig. 2. Influence of polyacrylamide molecular weight (106 Da) on the removal efficiency of Nannochloropsis oculata (A) and Chlorella vulgaris (C) [47] (reused with permission from Elsevier license number 5126300827044).

such study reported by Sun et al. [48] investigated the flocculation ability of poly(acrylamide-acryloyloxyethyl trimethyl ammonium chloride-butyl acrylate) (PADB). The cationic copolymer was a product of copolymerizing acrylamide, acryloyloxyethyl trimethyl ammonium chloride, and butylacrylate. It was applied on raw wastewater containing vast amounts of green microalgae. Harvesting efficiencies were determined from the extent of chlorophyll-a pigment removal from the suspension apart from turbidity analysis. PADB polymers displayed excellent flocculation abilities with increasing doses. PADB1 (20% cationicity) and PADB2 (40% cationicity) both offered optimum efficiencies for microalgae cells recovery at a low flocculant dose of 3 mg/L in the pH range of 6–8. Each removed 96.2% and 99.5% of chlorophyll-a pigments from the suspension, respectively. In addition, the lowest residual turbidities of 94.1% and 96.4%, respectively, were recorded under the same conditions. The high charge density variant PADB2 exceeded in performance. The efficiencies diminished beyond the optimum flocculant concentration for both the PADB variants. These observations reflect that with increasing doses, the cationic copolymers adequately neutralized microalgae cell surfaces and bridged between cells to generate large and dense flocs owing to their long polymeric chains. In the presence of excess flocculants, a charge reversal occurred at the microalgae cell surfaces that restabilized in suspension, in consequence, thereby decreasing the harvesting efficiencies. Moreover, PADB2 also outweighed a commercial cationic polyacrylamide bearing the same molecular weight but a higher charge density (35%). The flocculants removed 99.1% and 97.3% of chlorophyll-a pigments from the suspension at doses 3.5 mg/L and 3 mg/L, respectively [48].

Another study by Banerjee et al. [50] explored the flocculation efficiency of a polyacrylamide grafted starch flocculant in harvesting freshwater microalgae species *Chlorella sp*. The modified polymers exceeded in performance when compared to a starch flocculant. Grafted polymer grades with high grafting percentage possessed higher intrinsic viscosities and radius of gyration (reflecting expanded polymer configurations) in suspension that greatly enhanced their flocculation

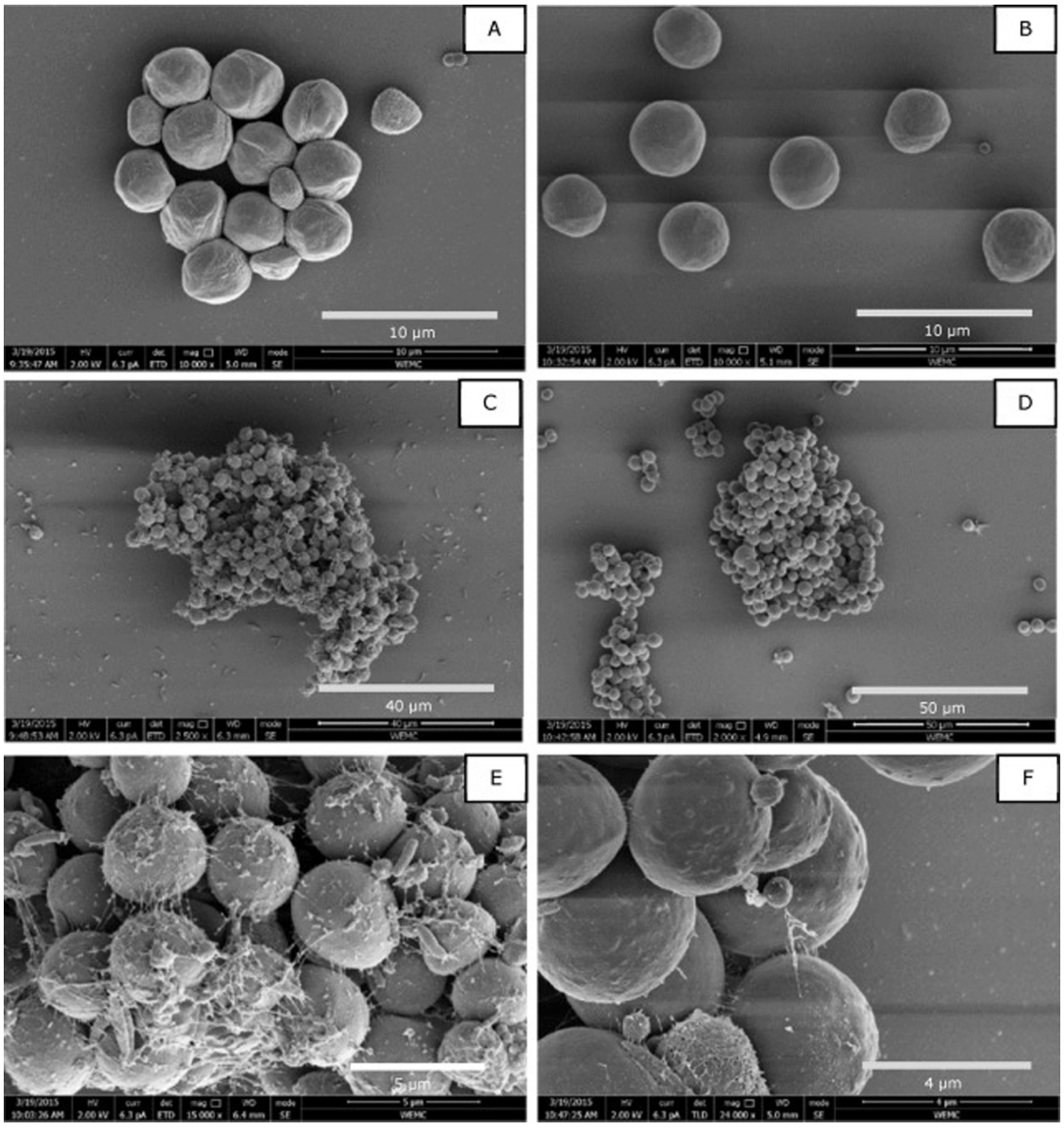

Fig. 3. SEM images for flocculation of marine microalgae *Neochloris oleoabundans* with cationic polyacrylamide Synthofloc 5080H under different salinity conditions. A: Microalgae cells without 5080H at 25 g/L salinity. B: Microalgae cells without 5080H at 45 g/L salinity. C: Microalgae cells flocculated with 5080H at 25 g/L salinity. D: Microalgae cells flocculated with 5080H at 45 g/L salinity. E: Magnified image of C to show bridges for 5080H at 25 g/L salinity. F: Magnified image of D to show bridges for 5080H at 45 g/L salinity. [36] (open access; permission to reuse not required).

efficiencies in microalgae suspensions. Flocculation efficiency was examined based on reduction in optical density. The flocculation behavior of the synthesized grades improved when the grafting percentage increased from 480% to 907%. Clearly, grafting polyacrylamide strands to starch flocculants tremendously enhanced their flocculation performance, while preserving their biodegradable properties.

In addition, the influence of pH on flocculation performance was also evaluated for the best performing grafted polymer grade St-g-PAM 2 (with the highest grafting percentage of 907%) at its optimum dose of 0.8 mg/L. Maximum reduction in optical density of the supernatant was observed to be 74% at pH 10.5. This coincided with the highest percentage of microalgae cells recovery of 85.84%. This can be explained by inferences obtained from previous studies that basic medium pH beyond 10 alone is sufficient to induce microalgae flocculation owing to salts precipitation and subsequent sweep flocculation [50].

In a novel attempt, Wang et al. [55] studied the synthesis of a magnetic polyacrylamide-based flocculant using iron oxide and 0.1 mg/mL of a cationic polyacrylamide. The resulting magnetic polyacrylamide was tested for its harvesting efficiency on cultures of two microalgae species, namely *Botryococcus braunii* and *Chlorella ellipsoidea*. The microalgae biomass was flocculated using a $Nd_2Fe_{14}B$ magnet positioned at the bottom of the Erlenmeyer flasks in which the cultures were flocculated. The permanent magnet was characterized by a magnetic induction intensity of 2000 G. The harvesting efficiencies tremendously improved with time from 2 to 10 min, reaching the maximum efficiency at 10 min. The harvesting efficiencies of the magnetic polyacrylamide exceeded 95% in 10 min for both the cultures at flocculant doses of 25 mg/L and 120 mg/L for the *Botryococcus braunii* and *Chlorella ellipsoidea* cultures, respectively. The massive difference in the flocculant doses may be accounted for by the small cell size of the *Chlorella ellipsoidea* microalgae that corresponds to a greater specific surface area, thereby requiring higher flocculant concentrations to attain a harvesting efficiency similar to the *Botryococcus braunii* culture.

A comparison of the flocculation efficiencies of the modified magnetic polyacrylamide against a conventional cationic polyacrylamide established the former more effective. Effective aggregation was observed in cultures flocculated with equal doses of both the polyacrylamide variants. Nevertheless, quicker sedimentation was observed for the flocs generated by the magnetic flocculant within 1 min under the influence of the permanent magnet. Flocs generated by the cationic polyacrylamide needed longer settling periods. Further, the harvesting efficiencies achieved using the cationic polyacrylamide were 57.33% and 84.45% for the *Botryococcus braunii* and *Chlorella ellipsoidea* cultures, respectively, over 30 min [55].

The medium pH was also noted to directly influence the magnetic flocculation process. The harvesting efficiencies for both cultures reduced as the medium pH was raised to 7, suggesting the suitability of lower medium pH for harvesting the *Botryococcus braunii* and *Chlorella ellipsoidea* cultures. The microalgae cells of *Botryococcus braunii* and *Chlorella ellipsoidea* species displayed negative zeta potentials over the pH range of 4–10. On the other hand, the magnetic polyacrylamide exhibited a positive zeta potential for medium pH below 7 and a negative zeta potential for medium pH above 7. Therefore, surface charge neutralization dominated flocculation mechanisms in the cultures when the medium pH was below 7, owing to the presence of electrostatic forces of attraction between the oppositely charged surfaces of the particles and the flocculants. In contrast, bridging flocculation was dominant when the medium pH was raised above 7 as both the cell surfaces and the magnetic polyacrylamide were negatively charged, ruling out any charge neutralization [55].

2.9. Flocs formation and characteristics for Polyacrylamide-Microalgae flocculation

An important parameter for determining the efficiency of a flocculant is the characteristics of the resultant flocs. Microscopic determination of floc characteristics reveals information about the compactness of flocs produced, indicated by the fractal dimension. Greater floc compaction is denoted by large fractal dimensions. This is highly desirable for flocs in wastewater treatment applications owing to the consequently high sedimentation rates, reduced sludge volumes and floc breakage on sludge pumping. Fractal dimension analyses were conducted by Sun et al. [48] for flocculation of green microalgae in raw wastewater using poly(acrylamide-acryloyloxyethyl trimethyl ammonium chloride-butyl acrylate) (PADB). The fractal dimensions for the produced flocs were monitored to increase with pH, from 1.262 at pH 3 to 1.298 at pH 7 and 1.358 at pH 11. The flocculant dosage also directly influenced the fractal dimensions. Improved fractal dimensions were recorded from 1.298 to 1.445 at pH 7 when the copolymer dose increased from 1.5 mg/L to 3 mg/L, respectively. These observations signify geometric restructuring within the irregular porous flocs with enhancing flocculation conditions.

Giraldo et al. [36] investigated the effect of salinity on the floc properties formed by microalgae when a cationic polyacrylamide, Synthofloc 5080H, was applied to harvest marine microalgae *Neochloris oleoabundans*. Scanning Electron Microscopy (SEM) technique was exploited to visualize the underlying adsorption and flocculation mechanisms exemplifying the microalgae-polyacrylamide interactions. Comparisons between microalgae media at 25 g/L and 45 g/L salinities were drawn. Fig. 3 (A) and 3 (B) revealed the state of microalgae cells in the absence of a flocculant at both salinities, respectively. While the cells are clustered, their surfaces appear smooth and are not interconnected by flocculant fibers. Fig. 3 (C) and 3 (E) depict microalgae media at 25 g/L flocculated by 60 mg/L of Synthofloc 5080H. Evidently, large amounts of cationic polyacrylamide strands are observed to effectively adsorb onto the cell walls of single microalgae cells owing to their stark charge differences. Moreover, these strands appear to interact with a group of cells, creating a fibrous flocculant network over clustered microalgae cells. Large and dense flocs were obtained in these conditions. This scenario clearly describes polymer adsorption and the combined involvement of surface charge neutralization and bridging mechanisms in the flocculation process. Similarly, Fig. 3 (D) and 3 (F) depict microalgae media at 45 g/L flocculated by 60 mg/L of Synthofloc 5080H. Though high salinity conditions also supported the formation of large microalgae flocs, Fig. 3 (F) exhibits a lower extent of polymer adsorption and the noteworthy lack of a fibrous system of flocculant amid microalgae cells. This case portrays polymer adsorption, prominent surface charge neutralization and hampered bridging interactions from polymer coiling induced by high salt concentrations [36].

You et al. [45] examined the floc properties for saltwater suspensions of microalgae *Chlorella vulgaris* flocculated with a commercial high molecular weight cationic polyacrylamide. At an applied flocculant dose of 1 mg/L, the polyacrylamide effectively aggregated microalgae cells within 8 min of the process and a considerable growth in floc size was recorded. The highest floc size obtained was 210 μm. A floc growth rate of 25.4 μm/min was computed for the cationic polyacrylamide. Beyond this point, a steady-state was achieved that represented a balance between floc growth and breakage. The high growth rate and large floc size are an outcome of the combined effects of charge neutralization and bridging mechanisms. Further, floc breakage analysis was also performed by altering stirring speeds from 50 rpm to 200 rpm. An instantaneous decline in floc size was noticed over 5 min and the final floc size was reduced to 30 mg/L. Successively, a floc regrowth analysis was conducted by reducing the stirring speed from 200 rpm to 50 rpm. Slight tendencies of regrowth were noticed for the flocs. However, the steady-state floc sizes could not be reached. A floc strength factor of 14.3 was calculated for cationic polyacrylamide-based microalgae flocs, indicating the formation of compact flocs and a high anti-shearing ability. Substantial floc sizes and floc growth rates demonstrate the suitability of cationic polyacrylamides as flocculating agents for microalgae suspensions in flocculation facilities [45].

2.10. Effect of microalgae species on flocculation

The harvesting efficiency of a polyacrylamide varies when applied to different microalgal species. For instance, Vermuë et al. [35] explored the harvesting efficiency of two cationic polyacrylamides Zetag 7557 and Synthofloc 5080H on two marine microalgae cultures of diatom Phaeodactylum tricornutum and green algae Neochloris oleoabundans. The harvesting efficiencies of the two polymers for Phaeodactylum tricornutum at a fixed flocculant dose of 10 ppm were recorded as 98% and 93%, respectively. However, the efficiencies significantly declined to 52% and 36%, respectively when the same polymers were applied to Neochloris oleoabundans cultures at the same dose.

Labeeuw et al. [40] expressed the effect of microalgae cell size on flocculation through the application of cationic polyacrylamide FO 3801 (high charge density and high molecular weight) to cultures of microalgae species *Chlorella vulgaris* (eukaryotic freshwater microalgae), Phaeodactylum tricornutum (eukaryotic marine microalgae) and *Synechocystis sp*. (prokaryotic cyanobacterium). FO 3801 was less effective as a flocculant when applied to eukaryotic microalgae species *Chlorella vulgaris* and *Phaeodactylum tricornutum* in comparison to prokaryotic microalgae *Synechocystis sp*. This could be attributed to the differences in cell sizes and the resulting cell surface areas accessible to the polymer chains for adsorption. The prokaryotic *Synechocystis sp*. species has the smallest cell diameter ~ 2 μm. Therefore, a high flocculation efficiency of 98.9% was achieved at a smaller optimum flocculant dose of FO 5 mg/L. The eukaryotic *Chlorella vulgaris* has a cell diameter in the range of 2–10 μm and the *Phaeodactylum tricornutum* species have the largest cell diameters of ~ 10 μm. Higher flocculant doses are necessary to achieve better surface adsorption on microalgae cell surfaces for effective charge neutralization and subsequent aggregation [40].

Mikulec et al. [31] also reported the effect of microalgae cell sizes and shapes on polyacrylamide-microalgae flocculation. Five microalgae cultures were analyzed, namely *Chlorella sorokiniana* (spherical-shaped green microalgae), *Parachlorella kessleri* (spherical-shaped green microalgae), *Scenedesmus obliquus* (spindle-shaped green microalgae), *Scenedesmus subspicatus* (spindle-shaped green microalgae) and *Synechococcus nidulans* (spindle-shaped blue-green microalgae). High molecular weight and high charge density cationic Sokofloc polyacrylamides 55GP, 57GP and 61GP employed. The highest flocculation efficiencies exceeding 95% were obtained for all cationic polyacrylamides for the spindle-shaped microalgae species *Scenedesmus obliquus, Scenedesmus subspicatus* and *Synechoccocus nidulans* in the lag growth phase at low flocculant doses ranging from 2 to 5 mg/L. This can be attributed to the large cell sizes and surface areas provided by these microalgae species for polymer adsorption in contrast to the spherical-shaped microalgae cells of *Chlorella sorokiniana* and *Parachlorella kessleri* that were two to three times smaller in size. The spindle-shaped cells were also able to form colonies that further enhanced microalgae cell removal from suspensions [31].

2.11. Effect of microalgae growth phase on polyacrylamide flocculation

Microalgae cells go through significant variations over successive growth stages in cell morphology, composition and structure of cell wall, intracellular substances and surface charges due to extracellular polymeric substances bound to cell surfaces. For biodiesel applications, targeted metabolites like lipids are commonly produced between the exponential and stationary growth stages of microalgae [56]. These growth stages are also of particular interest in polymer-based flocculation processes since many studies have presented high biomass recoveries at low flocculant doses during these stages. Hence, the impact of microalgae growth phases on flocculation efficiency must be examined.

The flocculation behavior of some polyacrylamides was notably affected by the different phases of microalgae growth. Labeeuw et al. [40] reported the impact of microalgae growth phases on the flocculation efficacy of two high-charge density and high-molecular-weight cationic polyacrylamides FO 3801 and FO 4808 in cultures of microalgae species *Chlorella vulgaris* (eukaryotic freshwater microalgae), *Phaeodactylum tricornutum* (eukaryotic marine microalgae) and *Synechocystis* sp. (prokaryotic cyanobacterium). The highest flocculation efficiencies above 90% at feasible flocculant doses ranging from 25 mg/g to 35 mg/g were achieved only until the stationary phase was reached for all the flocculants and microalgae cultures. The impact of various growth phases was not manifest on the flocculation efficiencies of FO 3801 and FO 4808 in the prokaryotic *Synechocystis* sp. cultures. Flocculation efficiency exceeded 95% within the dose range of 22.7–31.25 mg/g for both flocculants. Maximum efficiencies of 98.9% and 98.5% were obtained for FO 3801 and FO 4808, respectively, in the stationary phase.

Discernable distinctions in flocculation performances with growth stages were drawn in the cultures of eukaryotic microalgae. FO 3801 exhibited a low flocculation efficiency of 59.2% at a very high dose of 277.8 mg/g when applied to *Chlorella vulgaris* cultures in their early exponential phase. This increased sharply to 92.3% at a dose of 96.9 mg/g as the culture transitioned into its late exponential. Similar efficiency was achieved at a much lower flocculant dose of 34.5 mg/g in the stationary phase. The maximum efficiency of FO 3801 in the stationary phase was recorded at 99% at a dose of 53 mg/g. Further, FO 4808 (possessing a different N-substitute from FO 3801 in its polymer backbone) displayed poor flocculation efficiency of 64.2% at an unreasonably high dose of 833.3 mg/g when applied to *Chlorella vulgaris* cultures in their early exponential phase. Improved efficiency of 90% attained at a considerably lower dose of 110.7 mg/g as the culture transitioned into its late exponential. The maximum efficiency of 99% was attained at a dose of 26 mg/g only until the stationary phase was reached [40].

The effect of the microalgae growth phase on flocculation was further aggravated in marine eukaryotic microalgae *Phaeodactylum tricornutum* cultures. Practically no flocculation occurred when FO 3801 was added to the cultures in their early exponential phase. Satisfactory flocculant performance was observed in the late exponential phase when a flocculation efficiency of 82.3% was attained within the dose range 24.7–37.0 mg/g. Maximum efficiency exceeding 90% was obtained at a dose of 35.7 mg/g only until the stationary phase was reached. Moreover, FO 4808 could not achieve flocculation efficiencies above 50% even for a high dose of 200 mg/g in the early exponential phase of *Phaeodactylum tricornutum* cultures. Slightly higher efficiency of 77.5% attained at a significantly lower dose of 12.4 mg/g as the culture transitioned into its late exponential. Maximum efficiency exceeded 90% at a dose of 23.8 mg/g only until the stationary phase was reached [40].

2.11.1. Effect of algogenic organic matter released during various growth phases

Microalgae cells exude organic matter during their different growth phases. The secretions of metabolites, generally termed algogenic organic matter (AOM) or extracellular polymeric substances (EPS), can interfere with the polymeric flocculation process. EPS bound to microalgae cell surfaces like polysaccharides and proteins change the cell surface properties and affect the exposure of microalgae cell surfaces to polymer adsorption [40]. The secreted EPS may either be found attached to cell walls of microalgae cells or dissolved in water [57]. EPS attached to cell walls facilitate the formation of a network structure to uphold stable floc structures, particularly when inorganic coagulants like metal salts are employed for flocculation. Conversely, water-soluble EPS may neutralize cationic flocculants applied to microalgae suspensions. For instance, negatively charged carboxyl groups on polysaccharide chains interact with functional groups on cationic polyelectrolytes, thereby interfering with the microalgae-polymeric flocculant interactions that induce flocculation [58,59]. A few studies have established adverse effects of EPS on polymeric flocculation that surpass the impact of high ionic strength [37,59]. In several cases, the presence of EPS hampered effective flocculation by polymers for freshwater and marine microalgae

cultures and the required flocculant doses rose 7 to 9 fold [37,59,6049].

Mikulec et al. [31] explored the impact of secreted algogenic organic matter on the flocculation behavior of polyacrylamides for five microalgae cultures, namely *Chlorella sorokiniana, Parachlorella kessleri, Synechococcus nidulans, Scenedesmus obliquus,* and *Scenedesmus subspicatus*. A series of high molecular weight cationic Sokofloc polyacrylamides 55GP, 57GP and 61GP with charge densities of 20 mol%, 30 mol% and 55 mol% were utilized. The study revealed that all microalgae species released substantial amounts of dissolved organic matter into their culture media by the end of the linear phase of growth. The amount of organic matter was doubled in the medium during the stationary phase of growth for all microalgae species, except for *Parachlorella kessleri*. The impact of this variation was promptly reflected by the optimum flocculant doses required in each stage to induce effective flocculation. For instance, optimal flocculation conditions in *Scenedesmus obliquus* and *Scenedesmus subspicatus* suspensions using 55GP polyacrylamide were achieved at flocculant doses 0.5 mg/L in the linear growth stage and 2 mg/L in the stationary stage of growth viz. a four-fold increase in the required dose. The flocculation efficiency of 55GP was also adversely affected in *Chlorella sorokiniana, Parachlorella kessleri* and *Synechococcus nidulans* cultures by the high concentrations of dissolved organic matter in suspension [31].

The negative impact of algogenic organic matter on flocculation was further corroborated by removing the organic content from the cultures, resuspending the microalgae cells and subsequently introducing the cationic polyacrylamides to induce flocculation. This procedure led to dramatic reductions in optimum doses and consequent consumption of polyacrylamides alongside rapid sedimentation of substantial flocs. The occurrence of small concentrations of dissolved organic matter in the linear growth stage results in low flocculant consumption for inducing surface charge neutralization on microalgae cells. As the microalgae cell growth transitions towards the stationary phase, the concentration of dissolved organic matter increases. Polymeric substances like polysaccharides are typically present in algogenic organic matter. The negatively charged carboxyl groups on their chains neutralize cationic flocculants introduced into the suspensions and inhibit polymer-microalgae cell interactions. This phenomenon accounts for the increased flocculant volumes needed to reach optimum flocculation conditions. The effect is aggravated in the presence of cationic polyacrylamides possessing low to medium charge densities [31].

The efficiency of four high molecular weight cationic polyacrylamides FO 4450, FO 4650, FO 4800 and FO 4990 at a fixed flocculant dose was analyzed by Garzon-Sanabria et al. [37] on marine microalgae *Nannochloropsis salina* cultures in the presence and absence of algogenic organic matter. The polyacrylamide dose was fixed at 3 mg/L for cultures free from algogenic organic matter. Efficiencies in the range of 70–95% were achieved for all the polyacrylamides. Subsequently, flocculation tests of *Nannochloropsis salina* cultures were conducted in the presence of algogenic organic matter. A striking increase by sevenfold in the required flocculant dose (20 mg/L) was observed to achieve flocculation efficiencies similar to cultures free from algogenic organic matter. This study, therefore, ascertains that algogenic organic matter impedes the flocculation efficiency of polyacrylamides in marine microalgal suspensions. Based on the concentration assessment of total soluble proteins and carbohydrates secreted by the microalgae in the growth medium, it was estimated that the carbohydrates were more prone to impede the flocculation process due to their exponentially high concentration (100 mg/L) in the 7-days culture compared to proteins (4 mg/L) [37].

2.12. Effect of increasing cell density on polyacrylamide flocculation

Numerous studies have examined the significance of microalgae cell concentration in cultures flocculated with cationic polyacrylamides. According to Eldridge et al. [34], the microalgae cell recoveries improved with polymer dosage for suspensions with higher cell density. At the optimum dose of 3 mg/L and 5 min settling time for Zetag 7570, the efficiency of recovered cells rose remarkably from 74.1% to 92.4% when the initial microalgae cell concentration was increased from 0.29 g/L to 0.34 g/L. Aged cultures offered higher cell densities that enhanced flocculation kinetics by increasing cell collision rates. Therefore, lower flocculant dosages were required to achieve similar efficiencies for the two cultures [34].

Similarly, Labeeuw et al. [40] reported significant decreases in optimal flocculant doses for high molecular weight polyacrylamides FO 3801 and FO 4808 applied to high-concentration stationary phase suspensions of *Chlorella vulgaris* and *Phaeodactylum tricornutum*. Increased cell concentration intensifies intercellular collisions that enhance flocculation performance. Commercial polymeric flocculants have been reported previously to exhibit almost no effective flocculation in dilute microalgae mediums [61].

Further, König et al. [49] presented the highest harvesting efficiencies for *Conticribra weissflogii* cultures when the microalgae cell concentration was increased in the suspensions. The efficiency for high charge density and high molecular weight cationic polyacrylamide FO 4240 SH improved from 32.5% to 65% when microalgae cell concentration was raised from 1.2×10^5 cm^{-3} to 3.55×10^5 cm^{-3} at a flocculant dose of 4 mg/L. Increasing concentrations of microalgae cells in

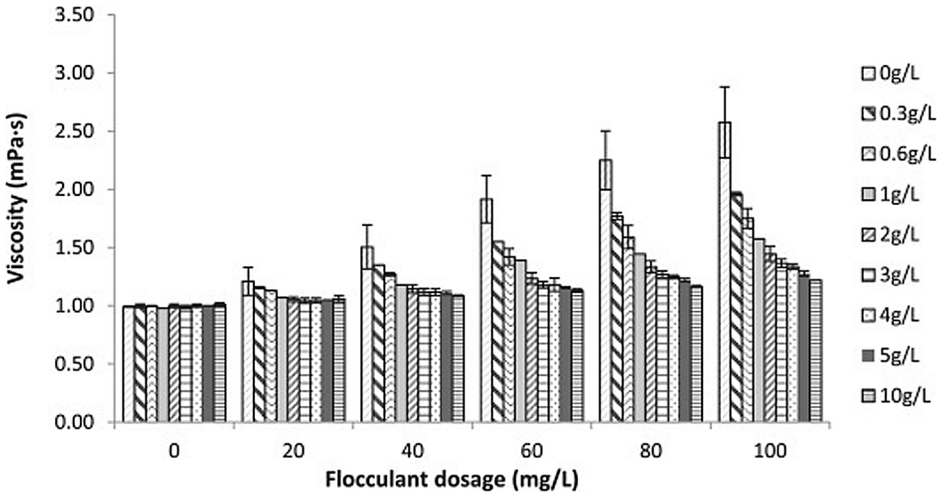

Fig. 4. Variations in cationic polyacrylamide Synthofloc 5080H viscosity (measured at a share rate of 100 s^{-1}) with increasing flocculant doses and salt concentrations [36] (open access; permission to reuse not required).

cultures diminish the intercellular distances promoting cell collisions and aggregation. Microalgae cell walls also possess lower surface charges in this condition [62].

2.13. Effect of medium salinity and ionic strength on Polyacrylamide-Microalgae flocculation

Dissimilarities in the leading flocculation mechanisms of polyacrylamides in freshwater and marine algae cultures were effectively demonstrated by Roselet et al. [47]. The effect of culture medium was very pronounced when a series of proprietary Flopam and Zetag polyacrylamides were employed to harvest microalgae from their cultures. While the polyacrylamide flocculants performed exceptionally well in freshwater microalgae *Chlorella vulgaris* suspensions, their flocculation behavior was inadequate in the marine microalgae suspensions. For instance, a polyacrylamide variant Zetag 8185 possessing a high molecular weight and low charge density was utilized to harvest *Chlorella vulgaris* and *Nannochloropsis oculate* microalgae species. At a fixed flocculant dose of 1.66 mg/L, 99% of *Chlorella vulgaris* algal cells were readily flocculated. On the contrary, the polymer unsuccessfully flocculated *Nannochloropsis oculate* algal cells at the same dosage, offering a poor harvesting efficiency of 15%. The high ionic strength of marine media induces coiling of polymer chains, thereby hindering bridging interactions for better flocculation performance. Decreasing the ionic strength of the medium was further noted to enhance the flocculation efficiencies of polyacrylamides on marine algae *Chlorella stigmatophora* by Bilanovic et al. [63]. In general, the maximum flocculation efficiencies recorded for all polyacrylamide variants ranged from 94 to 100% for flocculant doses varying from 45 mg/L to 1.66 mg/L, respectively, for freshwater *Chlorella vulgaris* suspensions. On the other hand, the maximum efficiencies noted for all polyacrylamides in marine *Nannochloropsis oculate* suspensions broadly ranged from 36 to 90% for flocculant doses varying from 45 mg/L to 0.55 mg/L, respectively [47].

Low harvesting efficiencies in marine media may be countered by using high charge density polyacrylamides. Giraldo et al. [36] examined the flocculation efficiency of a commercial high charge density cationic polyacrylamide Synthofloc 5080H on microalgae species *Neochloris oleoabundans* that grow in both freshwater and saltwater conditions. Salinity effects on the harvesting efficiencies were established at three concentrations of NaCl − 25, 35 and 45 g/L. High biomass recoveries beyond 90% were obtained under all conditions at an optimum dose of 30 mg/L based on optical density measurements. Flocculation is impeded beyond optimum dosage owing to restabilized suspensions.

The impact of salinity on Synthofloc 5080H was further established by viscosity measurements at increasing NaCl concentrations from 0 to 10 g/L [36]. It is well acknowledged that viscosity measurements indicate the configuration of polymer chains in aqueous solutions [64]. Fig. 4 clearly illustrates a systematic reduction in viscosity of Synthofloc 5080H with increasing salt concentrations, almost approaching the viscosity of water. This denotes the high sensitivity of the cationic polyacrylamide to the ionic strength of the medium. Reduced electrostatic repulsions within polymer chain segments at high ionic strengths lead to coiling of polymer chains. This deters bridging interactions between microalgae cells and polymer chain segments adversely affecting flocculation efficiency [65]. Nonetheless, the polyacrylamide still efficiently flocculates the microalgae suspensions as per optical density measurements. Zeta potential measurements were also conducted at two flocculant doses of 100 mg/L and 200 mg/L to address high flocculation efficiencies demonstrated by Synthofloc 5080H despite polymer coiling. Yet again, the zeta potential markedly decreased at both dosages when NaCl concentration was increased from 0 to 4 g/L in the medium, thereby confirming the outcomes of viscosity measurements [36]. It may be concluded that high flocculation efficiencies were preserved owing to the contribution of electric double layer compression around the negatively charged microalgae cells, in accordance with the DLVO theory [62].

A few studies have recounted the low sensitivity of certain commercial polyacrylamide flocculants to medium salinity. The influence of salinity on harvesting marine microalgal species *Nannochloropsis salina* with polyacrylamides was inspected by Garzon-Sanabria et al. [37]. Microalgae cultures free from algogenic organic matter were utilized in this study. Two concentrations of sodium chloride (NaCl), 5 g/L and 35 g/L, were tested. Negligible improvements in flocculation efficiencies in the range of 2–8% were observed with increasing ionic strength for the high molecular weight cationic polyacrylamides FO 4450, FO 4650, FO 4800 and FO 4990 at a constant dose of 4 mg/L. This indicated that the proprietary Flopam cationic polyacrylamides were less sensitive to high electrolyte concentrations in the medium. Slight improvements in recovery may be attributed to electric double layer compression around microalgal cells in the presence of electrolytes [37].

2.14. Effect of pH on Polyacrylamide-Microalgae flocculation

The influence of medium pH on the microalgae harvesting ability of polyacrylamides was examined by Uduman et al. [39]. In marine microalgae cultures of *Chlorococcum* sp., increasing medium pH coincided with improved harvesting efficiency for cationic (71303) and anionic (82230, Magnafloc 156) polyacrylamides. Increasing the pH to basic conditions for marine cultures triggers the salts in saline media to precipitate. In this case, it was noted that raising the medium pH above 10.5 itself sufficiently flocculated *Chlorococcum* sp. cells in the absence of polyacrylamides. Chemical precipitation of magnesium and calcium ions was induced at this pH that rendered salts like calcium carbonates, calcium phosphates, magnesium hydroxides, and calcium magnesium carbonates in the suspension [62]. These salts enhance the recovery of microalgae from suspensions via sweep flocculation [39].

Therefore, flocculation using the polyacrylamides was conducted at pH 4, 6 and 8, while the medium pH was originally around 8. The effect of pH was not very noticeable for cationic polyacrylamide 71,303 over changing medium pH, fundamentally due to the dominance of charge neutralization mechanism over the pH range. Minor reductions in efficiency from 89.9% to 86.5% were recorded when the pH was reduced from 8 to 4. In contrast, the influence of pH on flocculation was demonstrated when anionic polyacrylamides 82,230 and Magnafloc 156 were utilized. The recovery of microalgae diminished with reductions in medium pH to acidic conditions. Significant reductions in efficiencies were noted for 82,230 from 84.5% to 56.3%, and for Magnafloc 156 from 84.5% to 54.5% when the pH was reduced from 8 to 4. This is attributed to the increasing concentration of H^+ ions in the suspension that binds to the negatively charged functional groups on polymer chains, hampering bridging interactions and, subsequently, the flocculation efficiency. Correspondingly, zeta potential measurements were conducted for the flocculated microalgae suspensions to confirm the influence of altering medium pH. Notable reductions in suspension zeta potential were achieved when the pH was reduced from 8 to 4 as the zeta potential approaches the isoelectric point. A small decrease of 2.14 mV in suspension zeta potential was obtained for the cationic polyacrylamide 71303. In contrast, suspensions flocculated with anionic and non-ionic variants 82,230 and Magnafloc 156 displayed larger decreases in zeta potential by 3.04 mV and 5.28 mV, respectively [39].

Sun et al. [48] described the effect of pH alterations on microalgae containing wastewater samples flocculated with modified polyacrylamide copolymer poly(acrylamideacryloyloxyethyl trimethyl ammonium chloride-butyl acrylate) (PADB). Two variants PADB1 (20 mol% charge density) and PADB2 (40 mol% charge density) were utilized. Both chlorophyll-a removal and turbidity removal efficiencies increased rapidly when the pH was raised from 3 to 8. The highest turbidity removal efficiencies of 91.2% and 94.1% for PADB 1 and PADB 2, respectively, were obtained at pH 7. Maximum chlorophyll-a removal was achieved at pH 8 with 95.4% and 99.7% for PADB 1 and PADB 2, respectively. This can be explained by the abundance of H^+ ions in the medium in low pH conditions that neutralize and reverse surface

charges on microalgae cells. Simultaneously, H^+ ions also quaternize the PADB polymer chains in these conditions that generate electrostatic repulsions between the flocculant and microalgae cells. The cationic flocculants effectively neutralize the negatively charged microalgae cells under neutral pH conditions in the range of 6–9 and bridge between cells to promote flocculation. In contrast, raising the medium pH further from 8 to 11 resulted in sharp reductions in the flocculation efficiencies of the two flocculants. Chlorophyll-a removal efficiencies decreased to 78% and 83%, while turbidity removal efficiencies decreased to 67% and 75% for PADB 1 and PADB 2, respectively. While the negative charges on microalgae cell surfaces are preserved under high pH conditions, the polymer chains get hydrolyzed, adversely affecting their flocculation behavior [48].

Despite an evident impact of pH alterations noticed on the flocculation behavior of polyacrylamides in microalgae medium in previous studies, Nguyen et al. [44] reported minimum pH effects in the range of 6–9 for freshwater microalgae *Chlorella vulgaris* and marine microalgae *Phaeodactylum tricornutum* cultures. A high charge density and high molecular weight cationic polyacrylamide FO 3801 was applied at fixed optimum flocculant doses 18.9 mg/g and 13.7 mg/g to *Chlorella vulgaris* and *Phaeodactylum tricornutum* cultures, respectively, over the pH range. Negligible variations in optical density and zeta potential measurements were detected for each sample over the pH range, especially for freshwater microalgae samples. The flocculation efficiencies remained within 88.5–90.6% for *Chlorella vulgaris* while the zeta potential values altered from −5.12 mV to −5.64 mV with increasing pH over the range. On the other hand, a slightly elevated impact was detected for marine *Phaeodactylum tricornutum* cultures. The flocculation efficiencies varied over a broader range of 93.7–98.8% accompanied by zeta potential reductions from −3.41 mV to −1.63 mV with increasing pH. Since marine cultures are characterized by high ionic strengths, compression of electric double layer surrounding microalgae cells is anticipated. Nevertheless, this study illuminates the adaptability of cationic polyacrylamides for harvesting both freshwater and marine microalgae from their cultures over the studied pH range [44].

2.15. Application of flocculation aids with polyacrylamides

Polyacrylamides were also investigated for their harvesting performance for microalgae suspensions in combination with organic polymeric coagulants. Delrue et al. [38] reported exceptional flocculation performance by a set of commercial cationic polyacrylamides for harvesting *Chlamydomonas reinhardtii* microalga cultures. The polyacrylamide variant FO 4240 (15% cationic) exhibited a flocculation efficiency exceeding 95%. However, a dramatic reduction in flocculation efficiency to 20% was noted when FO 4240 was combined with polyDADMAC coagulant. Additionally, another combination of polyamine coagulant and an anionic polyacrylamide AN 910 (10% anionic) performed poorly and merely flocculated around 15% of the biomass [38].

An interesting investigation was conducted by You et al. [45] on the application of bentonite clay as a ballast agent to enhance the flocculation efficiency of a commercial cationic polyacrylamide in saltwater *Chlorella vulgaris* microalgae suspensions and reduce the associated costs. The use of high molecular weight cationic polyacrylamides alone rendered flocculation efficiencies up to 75% at a 10 mg/L dosage for a settling period of 20 min. Restabilization effects were detected beyond this concentration. Considerable decline in microalgae zeta potential was detected from −18.6 mV to −9.1 mV on the addition of 1 mg/L flocculant. Beyond this, no noteworthy reductions were noted. These results illuminate the major involvement of the charge neutralization mechanism in the flocculation process. However, the absence of any surface charge reversal effects coupled with large floc sizes implies the contribution of bridging interactions in the flocculation process as well [45].

On the other hand, the addition of small quantities of bentonite clay to the microalgae suspensions could dramatically enhance the harvesting efficiency of polyacrylamides. The application of 1 mg/L of cationic polyacrylamide in conjunction with an optimum bentonite concentration of 80 mg/L to *Chlorella vulgaris* suspensions offered a flocculation efficiency exceeding 94% at a significantly reduced settling time of 3 min. Further reductions in zeta potential were recorded. It must be noted that negatively charged bentonite particles are incapable of neutralizing the surface charges on microalgae cells. However, they can adsorb and bridge between microalgae cells to induce flocculation. Bentonite clays have characteristically high dilatability and specific surface area in water, thereby offering greater sites for microalgae adsorption and facilitate bridging interactions. Furthermore, when cationic polyacrylamides are added to stable microalgae suspensions, surface charge neutralization and some bridging interactions occur. When bentonite clay is added to this system consecutively, the negatively charged clay particles stick to the positively charged polyacrylamide patches on microalgae cells that contribute to further reductions in the microalgae zeta potential [45].

Floc characterization studies showed that the application of cationic polyacrylamides alone resulted in relatively small and loose flocs, which settled poorly over longer durations contributing to low flocculation efficiencies. Cationic polyacrylamides alone produced steady-state flocs within 8 min of the flocculation process that displayed a maximum size of 210 μm at 1 mg/L polymer dose beyond. On the other hand, the combination of 1 mg/L cationic polyacrylamide and 80 mg/L bentonite clay produced substantially larger steady-state flocs of maximum size 455 μm within 5 min of flocculation. The floc growth rates determined for the combination was 89.7 μm/min, almost four times higher than 25.4 μm/min for cationic polyacrylamide alone. The synergistic interplay of cationic polyacrylamide and bentonite clay are largely responsible for aggregating polyacrylamide-microalgae microflocs using bridging adsorption and electrostatic patch mechanisms. Floc breakage analysis revealed that the floc sizes reduced to 80 μm and 30 μm after 5 min for the combined flocculants and the polyacrylamide alone, respectively, when the stirring speed was raised from 50 rpm to 200 rpm. A larger floc strength factor of 17.6 was exhibited by the combination of flocculants than the cationic polyacrylamide alone, which presented a value of 14.3. This indicates that the flocs produced by the combination of flocculants displayed higher compaction and shear resistance. Further, flocs produced by cationic polyacrylamide coupled with bentonite presented a maximum floc recovery factor of 54.7 that was eight times higher than that for cationic polyacrylamide alone, which presented a value of 6.7 only. This suggests that bentonite particles efficiently bridged between the floc fragments exposed on floc breakage in the presence of electrostatic patches that enhanced the regrowth ability of the flocs [45].

2.16. Pilot-Scale and Large-Scale studies for Polyacrylamide-Microalgae flocculation

Pilot-scale investigations were conducted by Labeeuw et al. [40] in extension to lab-scale tests for determining the flocculation efficiency of a synthetic cationic polyacrylamide FO 3801 on cultures of three microalgae species, namely *Synechocystis* sp. (cynobacterium), *Chlorella vulgaris* (freshwater) and *Phaeodactylum tricornutum* (marine). The study revealed high flocculation efficiencies for polyacrylamide FO 3801 beyond a settling time of 10 min in microalgal systems of 350 L. Highest efficiencies were recorded at 87.5% for *Synechocystis sp.* at a dose of 44.6 mg/L, 82.7% for *Chlorella vulgaris* at a dose of 43.1 mg/L and 87.2% for *Phaeodactylum tricornutum* at a dose of 35.7 mg/L. Disintegration of flocs with time was also assessed by determining flocculation efficiencies after 24 h. Large flocs were found either sunken at the bottom or emerged to the surface, coinciding with increased efficiencies of 98.6% and 90.3% for *Synechocystis sp.* and *Phaeodactylum tricornutum* respectively. An exception to this trend was the *Chlorella vulgaris* suspension where large flocs had deteriorated with time, diminishing the

flocculation efficiency to 75.2% [40].

Another study conducted by Park et al. [46] tested the impact of continuously adding small doses of a commercial cationic polyacrylamide Crystalfloc B 490H to harvest vast microalgae colonies from a 1-hectare High-Rate Algal Pond utilized for domestic wastewater treatment. Predominant microalgae colonies identified in the wastewater pond included *Chlorella sp.* (~1–2 μm), *Desmodesmus sp.* (~10–20 μm) and *Dictyosphaerium sp.* (~10–40 μm). Laboratory scale tests were conducted to determine the optimum polyacrylamide dose at different mixing times and settling rates for effluent samples from the pond. The cationic polyacrylamide successfully reduced the turbidity by 70% at a small dose of 4 mg/L. A settling period of 15 min satisfactorily produced large and dense microalgae flocs. The optimal operational conditions were then extended to a hectare-scale algae wastewater treatment pond where the algal flocculation efficiency of the polyacrylamide was assessed over 21 days. The polyacrylamide was added continuously to the pond at the same dose rate for 14 days followed by a reduction to 2 mg/L over the next 7 days to examine the impact of reducing the flocculant dose on microalgae flocculation. Enormous improvements in the flocculation process were observed within three days echoed by a 40% reduction in turbidity levels in the algal pond. Overall, around 70% of the turbidity was reduced over 14 days. Further, decreasing the polyacrylamide dose over the last 7 days dropped the turbidity removal efficiency by 15%. Around 34 kg of flocculated microalgae biomass was harvested daily from the bottom of the algal wastewater pond using submersible pumps. Hence, small doses of high molecular weight cationic polyacrylamides can substantially enhance microalgae biomass recoveries from large-scale wastewater pond abundant in poorly-separable microalgae colonies [46].

3. Key challenges

3.1. Choosing an appropriate biomass recovery technology

A broad spectrum of technologies has been implemented for harvesting microalgae biomass from their cultures. Each method functions on its own principle, thereby presenting its own advantages and drawbacks. However, existing studies have been unable to singularize a specific technique or a combination of techniques as best adapted for harvesting various microalgae species. These techniques have been successfully applied to segregate microalgae biomass from their dilute cultures to a reasonable extent, primarily at the laboratory scale. Physical harvesting techniques have efficiently demonstrated the recovery of high quality and quantity of microalgae biomass in the absence of chemical additives. The range of techniques ensures minimum contamination of the recovered biomass and is greatly beneficial for deriving high-value products from the biomass. However, these techniques are marred by energy intensiveness alongside high associated capital and operational costs to be considered economically feasible for scale up [21,66].

In contrast, chemical harvesting techniques employ chemical additives as coagulating and flocculating agents to concentrate the microalgae biomass. The efficiency of the additives in aggregating the microalgae biomass is variable, depending on the type of chemical agent used. These harvesting processes outperform physical-based methods in terms of low energy requirements and techno-economic feasibility of large-scale production. However, the resulting quality and quantity of the biomass is relatively compromised and the biomass is contaminated with the chemical additives. This could produce a direct impact on the chemical profile of the harvested microalgae biomass and potentially lead to losses of valuable products. Similar features were observed for biological harvesting techniques, where the recovered biomass could be potentially contaminated with microbial species. Self-flocculation techniques for microalgae are not considered very reliable. Further, additional nutrients and sources with high organic carbon content are necessary to cultivate the microbial bio-flocculants in the medium. The symbiotic mechanisms between microbes (such as bacteria and fungi) and microalgae are also not fully understood [21,66].

Selecting a suitable harvesting technique or a combination of techniques requires careful considerations along the lines of the following criterions: the quantity of biomass to be harvested, the quality of the harvested biomass, cost-effectiveness, non-toxic nature of the process, duration of the harvesting process, and applicability to a wide variety of microalgae species and strains [67]. Coagulation-flocculation, centrifugation, filtration and flotation processes are mainly implemented for harvesting microalgae biomass selectively based on the industrial application. Flocculation processes are widely considered apt for harvesting microalgae biomasses for biofuels generation and restoration of water quality due to the low costs, standard operating procedures, and the ability to process large quantities of microalgae cultures. Filtration techniques are favored over centrifugation owing to their cost-effectiveness. They also outperform flotation techniques with respect to restoring water quality using microalgae biomasses owing to their high harvesting efficiency and lower associated harvesting costs for processing large amounts of water [16,20,67].

On the other hand, coagulation-flocculation processes are unsuitable against centrifugation, flotation and filtration techniques for harvesting microalgae biomass for food and feed applications. Centrifugation is chiefly preferred for producing dietary supplements for its high concentration factor, despite the high associated costs and energy demand [68]. The abovementioned physical harvesting techniques primarily ensure that the microalgae biomasses obtained are uncontaminated by chemical additives and the high-value products remain intact for extraction, both of which are compromised in the biomasses harvested by coagulation-flocculation processes [67,68].

Combining flocculation and filtration processes for harvesting microalgae exhibit tremendous potential with respect to generating high-value end products for pharmaceuticals, nutraceuticals and cosmetics. The combined process corresponds to low flocculant doses and reduced membrane fouling during the harvesting process, while offering a high-quality algal biomass. Flocculation effectively aggregates the free organic content in the culture, thereby significantly decreasing the chances of membrane fouling. Membrane filtration in conjunction with flocculation thus offers the highest membrane permeance and the least filtration resistance. Finally, the associated energy demand and processing costs are greatly reduced, in comparison to the individual processes, thereby reducing their drawbacks [69].

3.2. Lack of Large-Scale practical applications of microalgae flocculation

Compared to the traditional energy-intensive physical harvesting techniques, flocculation methods hold major advantages in terms of low operational costs and energy demand, versatile approach, high efficiency and adaptability to large-scale harvesting systems. A wide variety of flocculants have been studied for harvesting microalgae. Numerous synthetic and bio-based commercial organic polyelectrolytes have been successfully assessed for their harvesting efficiencies under small-scale laboratory conditions. However, there is a dearth of research studies extending the performance evaluation of these flocculants at pilot scale and industrial scale, particularly in outdoor mass culture systems. Based on a handful of pilot-scale and large-scale flocculation studies chiefly conducted using commercial polyacrylamides, it has become increasingly evident that the flocculation performances estimated for these flocculants from lab-scale jar tests can vary significantly from the performances determined in outdoor mass culture systems. This can be ascribed to variations in the existing physical and chemical conditions in the culture medium, including pH, solar irradiance, temperature, and dissolved oxygen [66]. Therefore, to prevent inconsistencies in the flocculation performance of these commercial polyelectrolytes on a larger scale, it is vital that flocculants are tested by imitating the same conditions during harvesting to ensure outdoor compatibility of the harvesting methods.

3.3. Harvesting marine microalgae with polyacrylamides

While freshwater microalgae species can be effectively harvested with cationic polyacrylamides at high flocculation efficiencies, harvesting marine microalgae from their cultures has been a major challenge for polyacrylamides as well as other effective bio-based polymers chitosan and cationic starch [24,30,70]. High salinity conditions in marine microalgae samples curb the flocculation process with polymeric flocculants. Effective flocculation performance may be achieved at salinity concentrations below 5 g/L [13]. The diminishing competence of cationic polyacrylamides to trigger microalgae flocculation in marine and brackish media is attributed to the coiling of polymer chains under high ionic strength conditions. The polymer chains shrink in conformation, thereby hindering the interaction of polymer active sites with microalgae cell surface and subsequent effective bridging between the suspended microalgae cells. The phenomenon is reflected by marked variations in the intrinsic viscosity of the polymeric flocculant in the medium [13,22]. The shielding effect induced by ions in marine media towards active sites on both the polyacrylamide chains and the microalgae surface also contributes to the reduced harvesting efficiencies [30].

Marine microalgae species are an attractive feedstock for omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). Those fatty acid groups have widely acknowledged benefits in the treatment of neuropsychiatric disorders (such as depression and dementia) and cardiovascular diseases [71,72]. Exploiting marine microalgae biomass for high-value products is further receiving attention as their large-scale cultivation does not require freshwater, reducing the pressure on existing freshwater sources. They thrive in saline water that is abundantly available [71,73]. Therefore, the development of an efficient and economically feasible harvesting process or a combination of processes for flocculating marine microalgae cultures is a promising field of research. Electro-flocculation has been considered as an alternative technology to harvest marine microalgae biomass. Low energy demand of the technique for treating marine cultures against freshwater cultures was reported, which arises primarily from the higher conductivity of marine media against freshwater media, raising the efficiency of the release of metal ions from the anode by electrolysis [71].

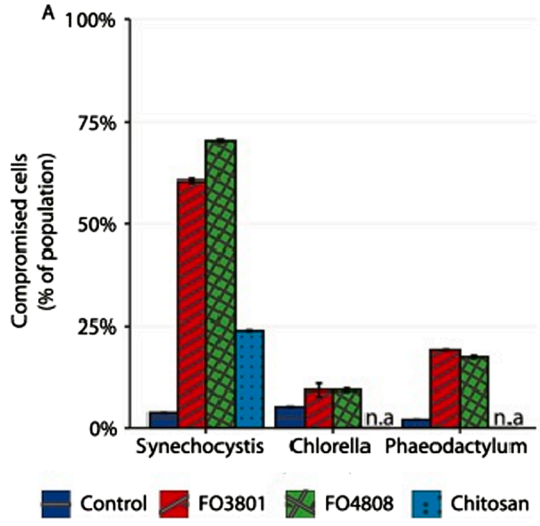

Fig. 5. The effect of two high charge density cationic polyacrylamides FO 3801 and FO 4808 and a chitosan polymer on the cell membrane integrity of microalgae species Synechocystis sp., Chlorella vulgaris, and Phaeodactylum tricornutum [40] (reused with permission from Elsevier license number 5126301405551).

3.4. Toxicity of polyacrylamide and acrylamide monomer

Polyacrylamide-based flocculants constitute the most important group of water-soluble polymers commercially applied in harvesting freshwater and marine microalgae from their suspensions. This is primarily due to the high reactivity, hydrophilic nature and relatively low costs associated with their monomer acrylamide [74]. While most polyacrylamide flocculants are stable and non-toxic, some may leave behind acrylamide residues that are anticipated to be neurotoxic, potentially carcinogenic and generally exhibit high toxicity towards aquatic life [47]. For instance, lethal concentrations (LC_{50}) of 411 mg/L and 119.5 mg/L were established for acrylamide in water based on acute toxicity tests conducted on Mediterranean mussel (*Mytilus galloprovincialis*) [75] and goldfish (*Carassius auratus*) [76], respectively, over 96 h.

Further, aquatic toxicity tests conducted on polyacrylamide flocculants state that cationic polyacrylamides are 100 times more toxic to aquatic life than the anionic variants, due to the presence of positively charged groups along the polymer chains. Cationic polymeric flocculants, in general, compromise the cell membrane integrity of the affected organisms. The effects may be exacerbated with increasing charge density and hydrophobicity of the polymer [30].

Nevertheless, most commercially applied high-molecular-weight polyacrylamides have presented great structural stability during treatment [23,40,77]. These industrial-grade polyacrylamides are widely recognized as safe polymers and have been successfully utilized in treating biological suspensions and drinking water [44,78]. These proprietary polyacrylamides have been recognized as non-toxic to humans, animals, and aquatic species, [77] and are readily hydrolyzed in water [44]. As a result, commercial polyacrylamides have found widespread utility in microalgae harvesting, industrial oily water treatment, potable water treatment, industrial and municipal wastewater treatment and sludge dewatering [74].

Moreover, with the ever-increasing endeavors targeting the use of sustainable flocculants, the non-biodegradable nature of polyacrylamides can be particularly unappealing. However, it is essential to note that the flocculation performances recorded for bio-based flocculants are not at par with polyacrylamides either in terms of harvesting efficiency or economic feasibility. Biopolymers like chitosan and cationic starch have been extensively studied due to their non-toxic and biodegradable nature. While a few studies have reported high harvesting efficiencies, the flocculants are expensive. Their flocculation performance is also affected by the medium pH. These features render their application in outdoor microalgae mass cultures unsuitable [21].

Despite its relatively higher toxicity, cationic polyacrylamides were best suited for efficient harvesting of microalgae biomass from large volumes of cultures at low flocculant doses, compared to the anionic and non-ionic variants. Anionic polyacrylamides perform inadequately and require very high flocculant doses to flocculate microalgae biomass. The application of flocculation aids such as inorganic and organic coagulants are necessary to achieve effective flocculation using these polyacrylamides. Non-ionic polyacrylamides are entirely ineffective in flocculating microalgae cultures.

Another significant advantage offered by polyacrylamides over other flocculants is the attainment of high flocculation efficiencies over a wide range of microalgae species and strains [30]. Several studies attempted at substituting polyacrylamide flocculants with non-acrylamide-based synthetic flocculants [79] and biopolymers such as modified chitosan and starch [10,21,22,80]. Nonetheless, an efficient alternative for polyacrylamides is yet to be achieved as the abovementioned flocculants were unsuccessful in matching up to the superior flocculation performance and cost-effectiveness of polyacrylamides.

3.5. Effect of polyacrylamide flocculants on cell membrane integrity

The quality of the microalgae biomass flocculated with

polyacrylamides was noted to be affected based on the type of microalgae species. Labeeuw et al. [40] tested the impact of two synthetic cationic polyacrylamides FO 3801 and FO 4808 possessing high charge densities and high molecular weights on three microalgae species, namely, cyanobacterium *Synechocystis sp*., freshwater microalgae *Chlorella vulgaris*, and marine species *Phaeodactylum tricornutum*. The cationic polyacrylamides were noted to considerably alter the integrity of the prokaryotic cyanobacterium *Synechocystis sp*. cells. The flocculants merely inflicted a minor impact on the eukaryotic species *Chlorella vulgaris* and *Phaeodactylum tricornutum*, in comparison to the unflocculated cells (Fig. 5). This implies that the cationic polyacrylamides can be effectively employed to flocculate *Chlorella vulgaris* and *Phaeodactylum tricornutum* with minimum effects on the cell membranes integrity and, by extension, the quality of biomass. However, they are unsuitable for harvesting prokaryotic microalgae species from their cultures. Since the intracellular components (such as lipids and proteins) are the target products in extraction, the flocculation techniques applied must have limited impact on the cells. It is essential to prevent any losses of the target components into the culture medium due to cell membrane disruptions.

Further, a comparison was drawn between the influence of the cationic polyacrylamides and a cationic bio-based chitosan polymer on the cell membrane integrity of the three microalgae species (Fig. 5). For the prokaryotic *Synechocystis* sp. species, the cell membranes of 60–70% cells were adversely affected by the cationic polyacrylamides at a flocculant dose 31.3 mg/g. On the other hand, a milder impact on the prokaryotic cells was recorded for chitosan. Around 23.6% of the cells were recorded with compromised membranes at a flocculant dose 375 mg/g. This indicates that the use of natural polymers is more suited for flocculating prokaryotic microalgae species, in contrast to synthetic cationic polyacrylamides bearing high charge densities. Furthermore, cyanobacteria species may contain toxins that would be discharged into the culture medium on cell disruption. This could prevent the reuse of the spent culture medium as a growth medium for cultivating microalgae.

The impact of cationic polyacrylamides on the cell membranes of the eukaryotic microalgae *Chlorella vulgaris* and *Phaeodactylum tricornutum* was far less. In comparison, only 14% and 19.3% of the cells for the two species, respectively, had compromised membranes when flocculated with the polyacrylamides. The optimum doses fixed for both the polyacrylamides were 34.5 mg/g and 35.7 mg/g for the two species, respectively. Chitosan failed to generate effective flocculation for the eukaryotic species. Hence, its impact on their cell membranes was not studied. Moreover, it was noted that the unflocculated biomass itself displayed 2–5.6% compromised cells, an indication of dying cells in the medium. Therefore, the actual number of cells compromised by flocculation with polyacrylamides is even lower.

The fundamental property responsible for the distinct structural integrities of prokaryotic and eukaryotic microalgae cell membranes is the composition of the cell membranes. The prokaryotic cyanobacterium *Synechocystis sp*. has a peptidoglycan-based cell wall and cell membrane, while the eukaryotic microalgae species *Chlorella vulgaris* and *Phaeodactylum tricornutum* have cell walls constituted by carbohydrates that provide structural reinforcement and improve their resilience to numerous environmental stresses [81,82].

Wu et al. [23] also studied the impact of a polyacrylamide flocculant on cells viability for eukaryotic microalgae species *Scenedesmus sp*. and *Scenedesmus obliquus* using an Evans blue assay protocol. Very few cells for both species were found to be affected, suggesting that the cell membranes for most cells remained intact. Therefore, the cationic polyacrylamide was not noted to inflict any significant damage during the harvesting process. Since the characteristics of the polyacrylamide were not provided, it is difficult to pinpoint the differences between the polyacrylamide flocculants used in this study and those discussed above. In addition, the physiological activity of the two types of microalgae cells was also analyzed after flocculation with polyacrylamide. The flocculated microalgae biomass for *Scenedesmus sp*. and *Scenedesmus obliquus* species was recultivated in fresh medium. Similarly, the microalgae biomass for the two species harvested via natural sedimentation was also recultivated in fresh medium. The growth performances observed for both approaches were nearly identical, further demonstrating the absence of cell lysis during flocculation. The impact of polyacrylamide flocculant on the photosynthetic apparatus was negligible. Future investigations are necessary over a wide range of microalgae species to positively determine the impact of polyacrylamides on the cell membrane integrity of the microalgae cells.

3.6. Effect of polyacrylamide flocculants on microalgae biomass composition

The effect of polyacrylamide flocculants on the composition of flocculated microalgae biomass was investigated by Labeeuw et al. [40]. Two synthetic cationic polyacrylamides FO 3801 and FO 4808 characterized by high charge densities and high molecular weights were used to harvest three microalgae species, namely, cyanobacterium *Synechocystis sp*., freshwater microalgae *Chlorella vulgaris*, and marine species *Phaeodactylum tricornutum*. The flocculation process evidently affected the chemical composition of microalgae cells. Notable changes in the metabolite composition were observed for *Synechocystis sp* and *Chlorella vulgaris*, while the composition of *Phaeodactylum tricornutum* cells was least affected. The two polyacrylamides also distinctly influenced the cell compositions despite being structurally similar. FO 3801 influenced the chemical composition of both Synechocystis sp. and *Chlorella vulgaris*. On the other hand, FO 4808 only altered the composition of *Chlorella vulgaris* cells. This feature can have a direct impact on downstream processing. Furthermore, despite the cell membrane integrity for *Chlorella vulgaris* cells being least affected by the polyacrylamides, the chemical profile of the cells varied starkly from the control. An overall change in the chemical profile of the cells may be attributed to the leakage of some metabolites into the culture medium due to compromised membrane integrity. However, no clear correlation between the cell membrane integrity results and the data for cell composition variation was attained in this study. This was particularly noted for the prokaryotic *Synechocystis sp*. biomass, where the polyacrylamides compromised the membranes of a large portion of cells. Nevertheless, only slight variations in the chemical compositions of the cells were observed from the control. Further examinations are necessary to determine whether the chemical profile variations arise primarily from leakage of metabolites during flocculation with polyacrylamides, or other factors also play a role.

3.7. Effect of polyacrylamide flocculants on the extraction of lipids

The impact of polyacrylamide flocculants on the quality of lipids extracted from the microalgae biomass was investigated by Borges et al. [83]. Two marine microalgae species *Nannochloropsis oculata* and *Thalassiosira weissflogii* were flocculated with an anionic (Magnafloc LT-25) and a cationic polyacrylamide (Flopam). While lipid profiles are species specific, the species under examination exhibited similar lipid profiles,

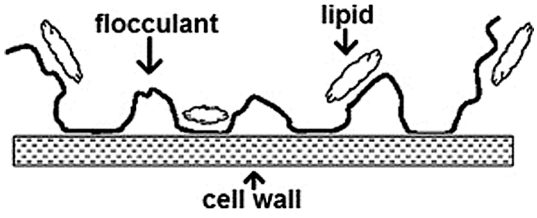

Fig. 6. Schematic illustrating the trapping of lipids by loops formed by a long-chain polymer flocculant adsorbed on to the microalgae cell wall [83] (reused with permission from Elsevier license number 5126310010049).

irrespective of the flocculant applied. For the *Nannochloropsis oculate* species, the control provided the maximum fraction of 4.3% for the lipids extracted from the biomass. The biomass was harvested by increasing the medium pH in the absence of a flocculant. Similar lipid fractions were obtained for cultures treated with the anionic polyacrylamide. On the other hand, treatment with the cationic polyacrylamide resulted in the least fraction of 3.6% for the lipids extracted from the harvested biomass. Similarly, for the *Thalassiosira weissflogii* species, the control resulted in the maximum fraction of 4.12% for the lipids extracted from the harvested biomass. On the other hand, the least lipids fraction of 2.77% was recorded for the treatment with the cationic polyacrylamide. In general, cationic polyacrylamides were noted to offer the lowest lipid fractions from the harvested biomass compared to other treatment methods. However, no considerable variations in the lipid fractions were reported over the range of treatments. These outcomes suggest that the polyacrylamides did not exert any significant influence on the lipid extraction process from the harvested biomass.

In contrast, considerable differences were noted in the fatty acid profiles of the two marine microalgae species when the two polyacrylamides were applied for flocculation, notably for the anionic polyacrylamides. The fatty acid profile of the control biomass for *Nannochloropsis oculate* exhibited the dominance of fatty acids C16:0, C16:1 and C20:5. Further, the major fatty acids found in the control for *Thalassiosira weissflogii* were C14:0, C16:0, C16:1, C16:3 and C20:5. On treatment with the polyacrylamide flocculants, significant variations in the fatty acids compositions were detected, in comparison to the control. For the *Nannochloropsis oculate* species, the fractions of C14:0 and C20:5 altered significantly with the application of the anionic polyacrylamide as higher concentrations of C14:0 and lower levels of C20:5 were detected against the control. Similarly, the addition of the anionic polyacrylamide to the *Thalassiosira weissflogii* cultures significantly reduced the concentrations of C16:0, C18:0 and C18:1n9t fatty acids when compared to the control. Cationic polyacrylamides also similarly decreased the concentrations of the same group of fatty acids, however, to a lesser extent.

Borges et al. [83] predicted that these variations in the fatty acid profiles triggered by the anionic and the cationic polyacrylamides arose from the interactions between the microalgae cell walls, the polyacrylamide flocculants, and the extracted lipids encompassing high concentrations of these specific fatty acids. In this scenario, the lipids extracted from the microalgae cells are trapped by the long-chained polyacrylamides adhered to the cell walls and enveloping a mass of cells. While the polyacrylamides interact with the cell walls via coulombic attraction, the extracted lipids get trapped by the polyacrylamide loops via London dispersion forces. The model postulated in the study (Fig. 6) delves into the possible interactions between the polyacrylamides and the extracted lipids. Depending on the type of polyacrylamide, some segments of the polymer chain adhere to the cell wall, while the other unabsorbed segments remain suspended, forming loops and tails. For anionic polyacrylamides, the active sites on the polymer chain weakly adsorb on the negatively charged microalgae cell wall at only a few sites due to dominant electrostatic repulsions. As a result, the polymer chains display a greater tendency to form loops that subsequently trap the lipids extracted from the cells. When lipids rich in certain types of fatty acids are trapped in loops, these fatty acids are not manifested in the fatty acids profiles for the studied microalgae species. This model can effectively illustrate why anionic polyacrylamides significantly altered the fatty acid profiles of *Nannochloropsis oculata* and *Thalassiosira weissflogii* microalgae species from the control, in comparison to cationic polyacrylamides that strongly adsorb on to the cell walls and present fewer occurrences of loops in the polymer chain.

This study reveals that the anionic and cationic polyacrylamides must be carefully selected for microalgae harvesting applications based on the intended products. When commercial polyunsaturated fatty acids such as Eicosapentaenoic acid (C20:5) or oleic acid (C18:1n9c) are products of interest, the application of polyacrylamides must be avoided for harvesting the microalgae biomass. Both types of polyacrylamides, particularly the anionic variants, were reported to cause significant reductions in concentration of these target products. In addition, the outcomes of this study also have implications with respect to biodiesel production. Biofuels predominantly composed of saturated hydrocarbons are generally more stable [84]. Lower concentrations of polyunsaturated fatty acids are desirable in biofuels mainly due to the higher susceptibility of these fatty acids to be oxidized. In contrast, the presence of higher levels of saturated fatty acids in biofuels impart greater resistance to oxidation and a high cetane number [85]. Therefore, using polyacrylamides for harvesting biomass can be beneficial for the production of biodiesel as the concentration of polyunsaturated fatty acids such as Eicosapentaenoic acid (C20:5) or oleic acid (C18:1) are dramatically reduced and the concentration of saturated fatty acids like myristic acid (C14:0) are enhanced during the lipid extraction process.

4. Conclusions and future prospects

Large-scale harvesting and application of microalgae biomass for biofuel generation still requires overcoming significant techno-economic barriers. Major efforts and investments in research and development are still necessary to achieve economic viability for the existing harvesting processes. Among the existing harvesting methods, no individual technique has been pinpointed as the optimum method for harvesting biomass of various microalgae species until now, necessitating future research and development initiatives on combining two or more techniques to optimize the harvesting process in terms of techno-economic feasibility, scalability, energy efficiency and sustainability.

Coagulation-flocculation processes have by far demonstrated greater efficiency, reliability, cost-effectiveness, versatility, and scalability in harvesting microalgae biomass over physical and biological harvesting methods. The performance of many synthetic and natural organic polyelectrolytes has been investigated in lab-scale studies for efficiently harvesting different microalgae species. Nevertheless, there is a lack of studies that demonstrate the high efficiencies for these flocculants under pilot-scale and-large scale conditions. Significant variations in flocculation efficiencies reported from a few studies conducted on pilot-scale and large-scale demonstrate the requirement of more upscaling studies in the future.

This review specifically emphasized on the performance of polyacrylamide-based flocculants applied for effective microalgae harvesting in existing literature studies. Several parameters affecting the flocculation efficiencies of these polyacrylamides were explored, which included the polymer properties such as the charge type, charge density, molecular weight and polymer architecture. High charge density and high molecular weight linear cationic polyacrylamides typically offered superior harvesting efficiencies in various microalgae cultures by diminishing the electronegativity of cell surfaces and bridging between the cells to bring about aggregation. Anionic and non-ionic polymers fail to produce similar effects primarily owing to electrostatic repulsions between cell surfaces and the negatively charged functional groups on the polymer chains.

Modifications on polyacrylamide chains are increasingly being attempted to enhance the harvesting efficiency, sustainability, versatility and recyclability of the generally successful flocculants. Magnetic polyacrylamides have also been synthesized and explored in efforts to combine chemical and magnetic flocculation, exhibiting promising results. A few studies have also analyzed the harvesting efficiencies of grafted polyacrylamides in microalgae cultures. Further research is required to develop novel grafted polyacrylamide-based flocculants. Successfully grafting polyacrylamides with bio-based flocculants (e.g. starch, polysaccharides) could enhance the biodegradability of the flocculants without compromising on the high flocculation performance owing to the presence of polyacrylamides [86]. The research prospect of developing recoverable and recyclable polyacrylamides is also highly endearing. This could facilitate not only the recovery of the applied

polyacrylamides in the culture but also recycling the supernatant from the medium for microalgae cultivation. The development of novel bio-based polyacrylamide variants could ensure that this cultivation medium does not negatively impact the microalgae cell growth, paving the way for more sustainable cultivation methods aside from the considerable cost reductions [87].

Moreover, the impact of the culture characteristics such as the type of microalgae species, growth phase of the culture, cell density, pH, salinity, and flocculation aids on microalgae flocculation with polyacrylamides was also analyzed. Highest flocculation efficiencies were attained in the stationary phase cultures of microalgae species. The release of large amounts of algogenic organic matter by microalgae into the culture was noted to reduce the polyacrylamide performance and render low biomass recoveries. High cell densities in microalgae cultures improved the flocculation performance of polyacrylamides. The pH of the culture influenced the flocculation performance by modifying the microalgae cell surface charge and the chain configuration of the polyacrylamides. High salinity conditions of marine microalgae cultures adversely affected the harvesting efficiencies. Alternative techniques like electro-coagulation or a combination of harvesting techniques must be explored and developed for effective harvesting of marine microalgae in an economically feasible manner.

Certain flocculants, including some polyacrylamides and biobased flocculants, have been noted to cause cell disruptions during the harvesting process. The effect was reportedly more prominent on prokaryotic microalgae species than eukaryotic microalgae species, affecting their cell integrity. Therefore, it is crucial to determine the type of microalgae species to be harvested by these polymeric flocculants to ensure an uncompromised biomass quality and retain the intracellular components of interest. It is also essential to identify alternative techniques for harvesting microalgae species that are sensitive to cell disruption by these flocculants to prevent any losses of the target components.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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