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Development of Innovative Flocculation Technologies for Agricultural Water Treatment

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DEVELOPMENT OF INNOVATIVE FLOCCULATION TECHNOLOGIES FOR AGRICULTURAL WATER TREATMENT

By

NOOR HALEEM

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

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2024

DISSERTATION ACCEPTANCE PAGE
Noor Haleem

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Dedication

This dissertation is dedicated to those whose generous support and encouragement have made this journey possible, particularly my parents and family.

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ABBREVIATIONS

CHPTAC - 3-chloro-2-hydroxypropyltrimethylammonium chloride 9

ANOVA - AAnalysis of variance 35

AGU - Anhydro glucose unit 34

BBM - Bold basal medium 58

CC - Cationic cellulose 51

CS - Cationic Starch 21

CCD - Central composite design 30

COD - Chemical Oxygen Demand 95

DS - Degree of substitution 21

DLVO - Derjaguin and Landau, Verwey, and Overbeek 12

DSC - Differential scanning calorimetry 51

DMSO-d6 - Dimethyl sulfoxide-d6. 34

DAB - Dry algal biomass 58

EDL - Electric double layer 18

EF - Electro-flocculation 18

T_g - Glass transition 57

GTAC - Glycidyl trimethyl ammonium chloride 27

HDO - Hydrogen deuterium oxide 64

FT-IR - Fourier transform infrared spectroscopy 56

IUPAC - International union of pure and applied chemistry 6

MO - Methyl orange 51

NMR - Nuclear magnetic resonance 51

PAC - Polyaluminum chloride 7

PPW - Potato peel waste 25

SEM	- Scanning electron microscope	33
RSM	- Response surface methodology	27
SS	- Suspended solids	21
TSS	- Total suspended solids	22
TN	- Total nitrogen content	45
VDW	- Van der Waals	16
ZP	- Zeta potential	14

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Abstract

DEVELOPMENT OF INNOVATIVE FLOCCULATION TECHNOLOGIES FOR AGRICULTURAL WATER TREATMENT

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The development of innovative flocculation technologies is essential for addressing the challenges of agricultural water treatment. These technologies play a crucial role in removing contaminants such as suspended solids and nutrients, thereby ensuring safer water for irrigation and livestock consumption. By enhancing water quality and wastewater management, they contribute significantly to environmental sustainability and public health in agricultural communities. This comprehensive thesis extensively explores various dimensions of flocculation, with a focused effort on methodologies and resources aimed at strengthening sustainability and efficiency.

A pivotal aspect of the research involves synthesizing cationic starch (CS), a flocculant derived from an underutilized resource, potato peel waste (PPW), thus supporting a circular economy (Ag-back to Ag-process). Through precise etherification with (3-chloro-2 hydroxypropyl) trimethylammonium chloride (CHPTAC), a water-soluble cationic starch with a high degree of substitution (DS) is successfully achieved. Optimizing reaction parameters yields promising results, with substantial reductions in total suspended solids (TSS) observed in swine and dairy wastewater samples. The study also focuses on preparing another natural flocculant, cationic cellulose (CC), achieved by etherifying commercial cellulose with CHPTAC, presenting a promising alternative for agricultural water treatment. Rigorous characterization techniques validate the successful cationization process, with the optimized CS & CC sample demonstrating exceptional performance across various environmental applications, including significant advancements in algal harvesting and dye removal processes. Furthermore, the thesis introduces a DC-initiated flocculation technology designed specifically for microalgal harvesting, aiming to overcome inherent limitations (contamination and cost) in chemical flocculants. By utilizing a DC electric field across titanium plate electrodes, this method achieves unprecedented efficiency in microalgal cell aggregation without the need for foreign chemicals. The notable contrast with traditional electrocoagulation methods lies in the utilization of inert electrodes, effectively bypassing the introduction of Al^{+3} ions into the fresh algal biomass which poses challenges, especially in the pharmaceutical and cosmetic industries. The promising scalability of this innovative approach offers significant potential for widespread adoption in both algae harvesting and processing, further solidifying its status as a cornerstone of sustainable agricultural water treatment practices. Ultimately, this study serves as a beacon of hope that highlights the transformative impact of innovative flocculation technologies in agricultural water treatment. By promoting environmentally friendly alternatives and embracing circular economy principles, these advancements have the potential to revolutionize agricultural water management with sustainability, efficiency, and environmental stewardship.

Chapter 1

Introduction and Background

1.1. Agricultural water & wastewater: Generation and treatment overview

The growing demand for food production driven by a projected global population of 9.7 billion by 2050 (Vaishnav et al., 2023), has intensified agricultural activities. However, these essential practices, including farming, irrigation, and livestock watering, often result in the generation of substantial amounts of agricultural wastewater. This type of wastewater consists of excess water from surface irrigation, effluent from processing field crops containing fertilizers and pesticides, and discharge from facilities like swine, dairy, and poultry farms. Characterized by high levels of organic matter, chemical oxygen demand (COD), and total suspended solids (TSS), agro-industrial wastewater poses significant pollution risks if released untreated into the environment (Kumar et al., 2021). Therefore, it requires chemical and biological treatment before discharge to mitigate its eutrophic and polluting potential.

The treatment of agricultural wastewater provides a solution to the global problem of water scarcity and environmental pollution. The industrial sector utilizes 22% of global freshwater, while agriculture accounts for 70% of withdrawals, yet only 24% of wastewater undergoes treatment before disposal (Lakshmi & Reddy, 2017). Livestock facilities further exacerbate this issue by flushing out pathogenic bacteria with large volumes of water, often mixing domestic wastewater with rainwater, resulting in the flow of substantial volumes of pathogenic wastewater. Various methods have been explored for removing contaminants from water, including construction wetlands (Rozema et al., 2016), membrane bioreactor (Cicek, 2003), coagulation-flocculation, adsorption, ion exchange, precipitation, membrane filtration, and electrochemical flocculation (S. Muhammad, Yahya, Abdul Khalil, Marwan, & Albadn, 2023). Commonly used physicochemical processes like coagulation-flocculation and adsorption are limited by the high cost of chemical usage. Biological methods, like combined activated sludge and biosorption techniques, are gaining attention for their sustainability but pose challenges in biomass management, operational costs, and treatment times (Ang & Mohammad, 2020). As waste management systems evolve to address global environmental challenges, there is a growing need to explore innovative agricultural water treatment approaches aiming to overcome current method limitations and enhance overall efficiency and sustainability.

1.2. The fundamental of coagulation-flocculation processes

Particle removal is a critical aspect of water treatment, especially when dealing with micron-sized particles, which pose significant challenges. Coagulation serves to destabilize colloidal particles, facilitating their agglomeration, while flocculation further aids in forming larger aggregates for easier separation. Coagulants typically possess a higher net charge and lower molecular weight compared to flocculants. To achieve effective flocculation, moderate agitation is recommended, as excessive agitation can disrupt the formation of flocs. The coagulation-flocculation process is essential in water treatment, effectively reducing turbidity, suspended solids, oil, organic matter, color, and COD in both water and wastewater. This process involves introducing substances that help cluster fine particles into larger flocs for removal from the water (Tzoupanos & Zouboulis, 2008). Coagulation neutralizes the negative charge of suspended particles, while flocculation physically forms flocs from suspended colloids using high molecular weight compounds such as starch, cellulose, and chitosan as shown in Fig.1.1 (Saritha, Srinivas, & Srikanth Vuppala, 2017). Coagulation initiates the formation of larger flocs, which then aggregate and settle during flocculation. Incomplete coagulation can result in ineffective flocculation and sedimentation. During treatment, the untreated water undergoes vigorous mixing to ensure uniform distribution of coagulants followed by slower mixing to facilitate floc bridging. Sedimentation allows larger solid particles to settle, and the treated water is often stored before use. However, prolonged storage of water rich in nitrogen, phosphorus, and potassium can lead to algae and microbial growth (Mehta, Khunjar, Nguyen, Tait, & Batstone, 2015).

Direct flocculation has emerged as a cost and time-saving method, employing medium charge density cationic polymers with high molecular weight. These polymers neutralize the negative charges of colloidal particles and bridge destabilized particles to form flocs (Chong, 2012). Unlike conventional coagulation-flocculation processes, direct flocculation does not necessitate additional coagulant or pH adjustment. Moreover, the use of high molecular weight polymers enables effective bridging of colloidal particles across various pH values, while also reducing sludge volume due to densely packed flocs (Lee, Robinson, & Chong, 2014). However, its application is primarily suitable for organic-based wastewater with high concentrations of suspended and colloidal solids effluents. Thus, conventional coagulation-flocculation processes remain preferred for agro-industrial wastewater, as they can treat both inorganic and organic-based wastewater. The selection between coagulation-flocculation and direct flocculation depends on the type of wastewater being treated. Despite its limited application, direct flocculation offers advantages such as reduced chemical usage, simpler processes, less sludge production, and lower treatment costs, making it a worthwhile option to explore further (Lee et al., 2014).

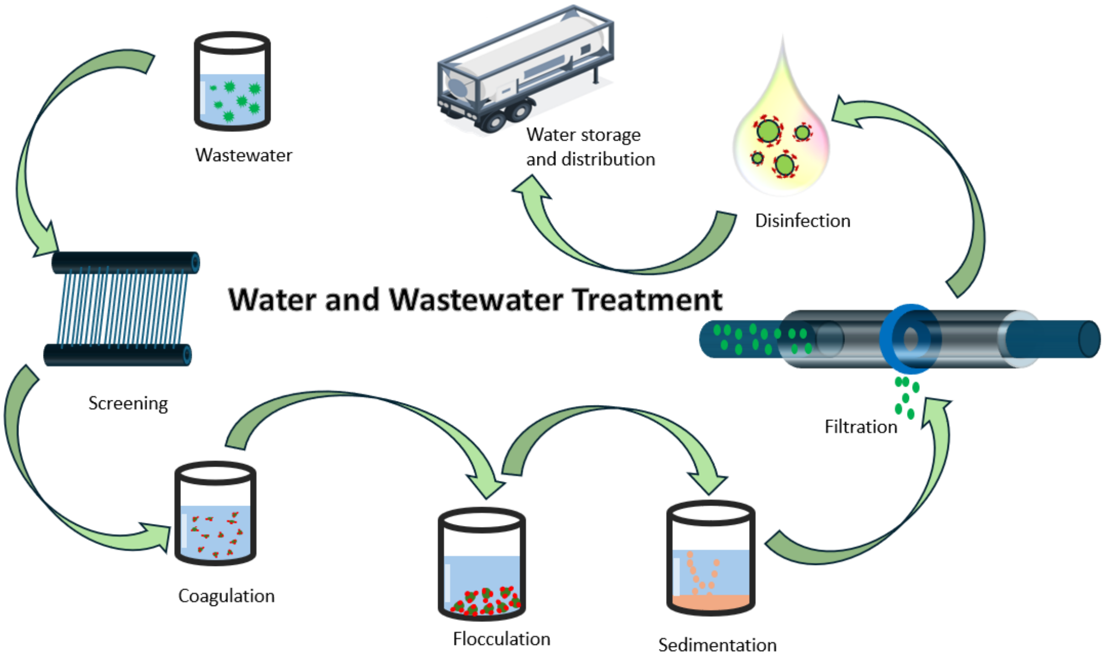

Figure 1.0 Various steps in the coagulation and flocculation processes for agricultural water and wastewater treatment.

Stages of flocculation

Perikinetio flocculation

Perikinetic flocculation, driven by thermal agitation or Brownian movement, is a rapid and random process that occurs immediately after destabilization. It typically completes within seconds due to a maximum floe size that limits the effect of Brownian motion (Fig.1.2). Although thermal kinetic energy overcomes the energy barrier between colloidal particles, this barrier increases as particles combine, eventually leading to perikinetic flocculation (Oyegbile, Ay, & Narra, 2016).

Orthokinetic flocculation

Orthokinetic flocculation occurs due to induced velocity gradients in the liquid. These gradients can be caused by various factors, such as liquid motion around baffles or mechanical agitation in a flocculation reactor. These velocity gradients create relative velocities between particles, facilitating opportunities for contact (Zhu, 2014).

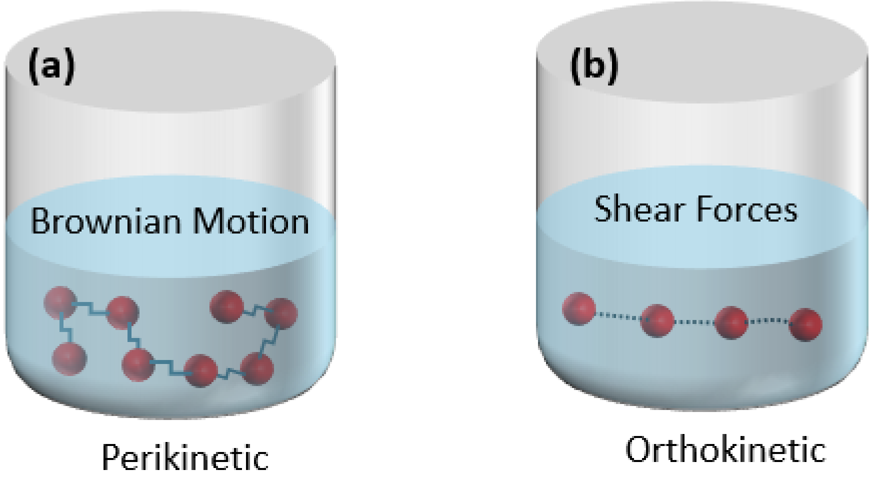

Figure 1.2. Interaction of Particles (a) Brownian motion: particles exhibit zigzag movement due to collisions with fluid molecules. (b) Shear forces: particles collide with layers of fluid experiencing shear forces.

Challenges in coagulation and flocculation processes

Challenges associated with the use of coagulants and flocculants in water treatment include various environmental and operational concerns.

1. The use of iron-based coagulants has been limited, potentially due to issues with color and oxidation-reduction reactions (Iwuozor, 2019).
2. Alum is the most used coagulant, but it carries the risk of corrosive problems in equipment due to sulfate compounds in the sludge (Iwuozor, 2019).

3. The use of inorganic coagulants has declined due to environmental consequences like the production of toxic metal hydroxide sludge and increased metal concentration in treated water, which can have adverse health effects (Teh, Budiman, Shak, & Wu, 2016).
4. Synthetic polymers offer advantages such as ease of use and effectiveness, but their higher cost compared to traditional coagulants limits their widespread acceptance (Lee et al., 2014)
5. Most synthetic polymers are non-biodegradable, or their degradability is usually slow, and their degraded products are considered hazardous because of the release of monomers that could enter the food chain and may cause carcinogenic effect (Vandermeulen, Boarino, & Klok, 2022).

To address these concerns researchers are prioritizing the development of natural-based polymeric flocculants, ultimately aiming to enhance water treatment processes while reducing environmental and health risks. This growing need for eco-friendly and efficient coagulant aids has led scientists worldwide to explore the development of natural flocculants derived from natural sources as potential substitutes for synthetic alternatives.

Coagulants & flocculants

In water and wastewater treatment, coagulants and flocculants play crucial roles, each serving specific functions. Despite their long history of being used interchangeably, the International Union of Pure and Applied Chemistry (IUPAC) does not provide strict definitions for these terms. However, conventionally, coagulants are recognized for their role in promoting aggregation, while flocculants are primarily responsible for facilitating the aggregation of particles (Mickova, 2015).

Coagulants: Coagulants are chemicals primarily used to destabilize colloidal and suspended particles in water. By neutralizing the electrical charges of these particles, coagulants facilitate their aggregation leading to the formation of larger and heavier particles known as flocs. These coagulants are typically added to water and wastewater in the early treatment process after the initial screening and primary settling stages, initiating particle aggregation and thereby facilitating the removal of impurities from the water (Jiang, 2015). There are two main types of coagulant chemicals: primary coagulants and coagulant aids. Primary coagulants neutralize the electrical charges of suspended solids in water, while coagulant aids increase the density of slow-settling flocs, making them more resistant to shearing during mixing and settling processes. Coagulant chemicals can be either inorganic metallic salts like aluminum sulfate (alum), ferric chloride, poly aluminum chloride (PAC), and various metal salts or polymers. Polymers come in three types: cationic (positively charged), anionic (negatively charged), and non-ionic (neutrally charged).

Flocculants: Flocculants play a crucial role in destabilizing colloidal particles by bridging them, a process facilitated by polymers acting as glue between the colloids. These polymers, mostly linear and water-soluble, are derived mostly from oil-based and nonrenewable raw materials with varying molecular weights. The widespread use of polymers as flocculants stems from their distinct characteristics including ease of use, immediate solubility in aqueous systems, and neutrality towards the pH of the medium. They are highly efficient, requiring only small quantities (e.g., a few milligrams per liter), and the flocs formed during flocculation are larger and stronger. Additionally, the use of an appropriate polyelectrolyte can further increase floc size, resulting in dense, well-formed flocs with good settling characteristics (P. Maćczak, H. Kaczmarek, & M. Ziegler-Borowska, 2020). Flocculants are also categorized based on their charge types, with each offering unique benefits such as high solubility, pH neutrality, and the efficient production of large, stable flocs. However, despite their advantages in water treatment, polymeric flocculants sourced from petroleum pose environmental and health risks due to their non-biodegradability and the potential carcinogenic effects of released monomers. However, certain variants, particularly anionic and nonionic types, exhibit lower toxicity rates compared to cationic ones (Okaiyeto, Nwodo, Okoli, Mabinya, & Okoh, 2016). Hence, there is a growing interest in environmentally safe alternatives like natural flocculants, which present a viable option for water treatment processes.

1.3. Development of natural flocculants

Sources of polysaccharides flocculants

Utilizing biomass-based raw materials in the synthesis of new bio-based flocculants aligns with the principles of green development, offering advantages such as being environmentally friendly, non-toxic, and biodegradable. These bio-based flocculants hold significant promise as alternatives to traditional organic synthetic flocculants due to their abundant natural reserves, ease of natural degradation, and high material safety standards (Salehizadeh, Yan, & Farnood, 2018). Polysaccharides serve as a noteworthy category of natural flocculants, primarily sourced from agricultural and forestry feedstocks. Examples include cellulose and starch, which are derived from agricultural and forestry sources, showcasing the versatility and sustainability of biomass-derived materials in flocculant synthesis.

Natural flocculants in agricultural water & wastewater treatment

The growing interest in exploring sustainable alternatives, particularly natural flocculants, for agricultural water and wastewater treatment reflects a shift away from conventional methods reliant on synthetic chemicals. Natural flocculants, derived from renewable sources like plants and microorganisms, show promise for purifying water & wastewater while minimizing environmental harm (Das, Ojha, & Mandal, 2021). Additionally, their ease of modification enhances their efficacy in flocculation, making them a cost-effective and environmentally friendly solution for water treatment (Piotr Maćczak, Halina Kaczmarek, & Marta Ziegler-Borowska, 2020). Despite the popularity of polymeric flocculants in industrial effluent treatment, which are valued for their pH stability, high efficiency, and ease of use, natural flocculants offer a promising eco-friendly alternative with distinct advantages and limitations. The effectiveness, sustainability, and scalability of these alternatives contribute to advancing environmentally friendly solutions for mitigating agricultural water pollution. However, natural flocculants are known for their moderate efficiency and relatively short shelf life due to the biodegradation of their active components over time, necessitating proper control to maintain stability and strength in formed flocs (Lee et al., 2014).

Cationic starch

Starch, a widely available and inexpensive polysaccharide, serves as a crucial carbohydrate storage product in plants found abundantly in cereal and tuber plants like maize and potatoes. Its natural granular form can be modified to dissolve in water, making it a versatile and renewable resource for various applications, particularly in the field of water treatment. Utilizing starch as a polymer for creating reactive cationic moieties, facilitated by quaternary ammonium compounds, offers several advantages. Its biodegradability and cost-effectiveness make it an ideal raw material for producing cationic flocculants (L. Wei, Jinju, Hongjian, Zongwu, & Xinding, 2021). By incorporating cationic monomers like N-(3-chloro-2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC), different grades of cationized starch (with different DS) can be synthesized, exhibiting effective flocculation properties in wastewater treatment (Fig.1.3). These cationic polysaccharides, containing positively charged groups from various reagents like amino, imino, ammonium, sulfonium, or phosphonium, demonstrate versatility and efficacy across different pH ranges (S. Pal, D. Mal, & R. P. Singh, 2005). Cationized starches effectively treat wastewater containing negatively charged organic and inorganic matter, serving as flocculant to precipitate suspended particles in water. Additionally, introducing cationic groups to starch enhances its mineral binding properties, crucial for anchoring minerals to fibers (Järnström, Lason, & Rigdahl, 1995).

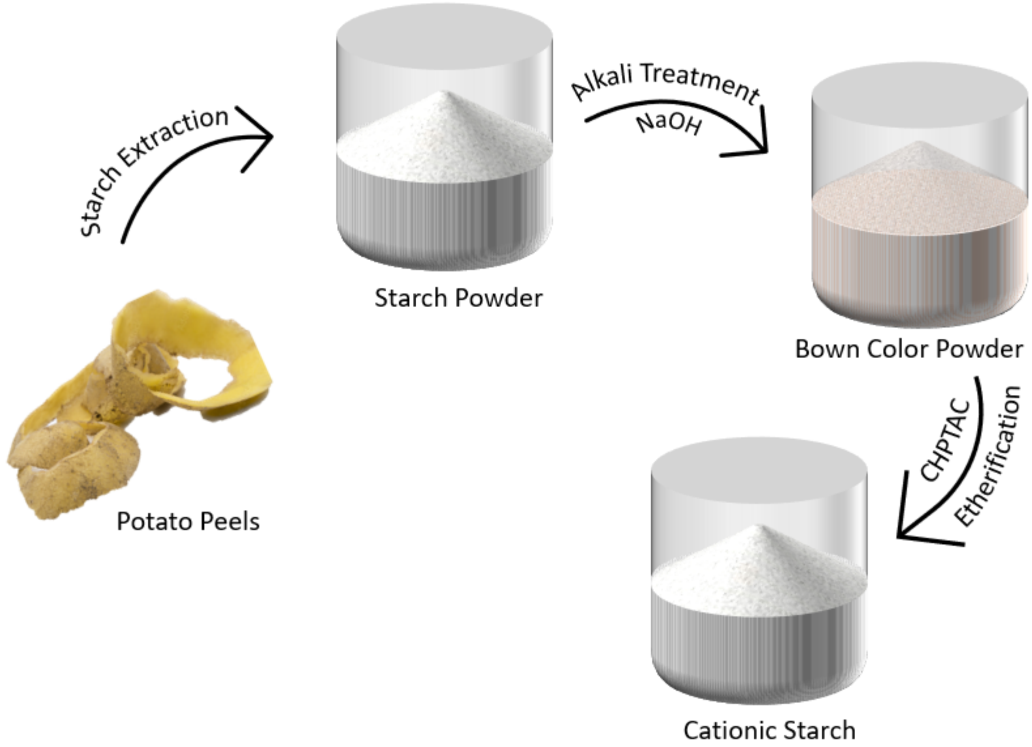

Figure 1.3. Process overview from potato peel starch extraction to cationic starch conversion.

Cationic cellulose

The renewable abundance of cellulose makes it a promising alternative for diverse applications in wastewater treatment, emphasizing its role as an eco-friendly option for developing natural flocculants and supporting sustainable environmental practices. Cationization of cellulose enhances its ability to destabilize suspended particles, aiding in solid-liquid separation in wastewater treatment. The reaction of chemicals like GTMAC and CHPTAC with the hydroxyl groups in cellulose introduces positively charged functional groups onto the cellulose backbone, facilitating the flocculation of suspended particles in wastewater. This process effectively removes various contaminants from wastewater streams, making it a promising method for producing flocculants (Gupta, Ladino, & Mekonnen, 2023).

Selection of flocculants

The selection of an appropriate flocculant is a critical step in treating wastewater to effectively remove suspended solids and contaminants. Initial assessment of wastewater characteristics guides the choice of treatment process. In agricultural water treatment, the composition analysis determines whether coagulation-flocculation or direct flocculation is the optimal method. This strategic approach ensures effective contaminant removal and minimizes environmental impact. Synthetic polymers (flocculants) are generally classified into four forms: cationic (positively charged), anionic (negatively charged), amphoteric (containing both cationic and anionic groups), and non-ionic (close to neutral). Since colloidal suspensions typically have a negative surface charge, cationic coagulants or flocculants are commonly chosen (Dayarathne, Angove, Aryal, Abuel-Naga, & Mainali, 2021). In the coagulation-flocculation process, cationic coagulants are commonly paired with non-ionic or anionic flocculants. On the other hand, for direct flocculation, cationic or anionic flocculants are usually chosen. High molecular weight flocculants are preferred in these processes because they offer a stronger bridging mechanism compared to other flocculation mechanisms (Lee et al., 2014).

1.4. Interaction mechanisms of particles in flocculation processes

The interaction between colloidal particles in water, influenced by attractive and repulsive forces, significantly affects sedimentation behavior and floc formation. Floc formation is a complex process involving the dispersion and diffusion of flocculants, their adsorption onto particle surfaces, particle collision, and the subsequent growth of micro-flocs. The type and dosage of flocculants are essential factors for achieving effective particle aggregation, which may be achieved through particle destabilization by double-layer compression or physical enmeshment of colloids within coagulant precipitates, and chemical reactions, or chemical sorption (Fig.1.4) (Lee et al., 2014). Adsorption mechanisms and the molecular weight of the flocculant further determine the size and structure of the resulting flocs (Taylor, Morris, Self, & Smart, 2002). Optimizing the optimal dosage of flocculant is critical during the flocculation process before sedimentation and filtration. Inadequate dosage can impede floc formation, while excessive amounts can elevate filtration resistance. This is because excessive flocculant can cause the formation of dense or compact flocs, which are more prone to clogging filtration systems and retaining water within the flock (Xia et al., 2022). Using different flocculants can improve the effectiveness of the treatment process by targeting a wider range of suspended particles.

The surface charge of solids also plays a significant role in their interaction, as described by the Derjaguin and Landau, Verwey, and Overbeek (DLVO) theory. This theory explains the strength of interaction between colloidal particles, which is governed by attractive van der Waals forces and repulsive electrical double-layer forces. Depending on the surface charge, particles may either stick together upon contact (low surface charge) or slide past each other (high surface charge) due to Brownian motion collisions (Alamgir, Harbottle, Masliyah, & Xu, 2012). These interactions influence the settling rate and sediment formation: low surface charge leads to fast settling and porous sediment with high moisture content, while high surface charge results in slow settling and compact sediment with low moisture content, high molecular weight polymeric flocculants generate larger, more open flocs, whereas low molecular weight ones produce smaller, denser flocs (Guo, Gao, Wang, Yue, & Xu, 2019). Precise flocculant dosage is essential, as insufficient amounts impede floc formation, while excessive doses elevate filtration resistance or moisture content.

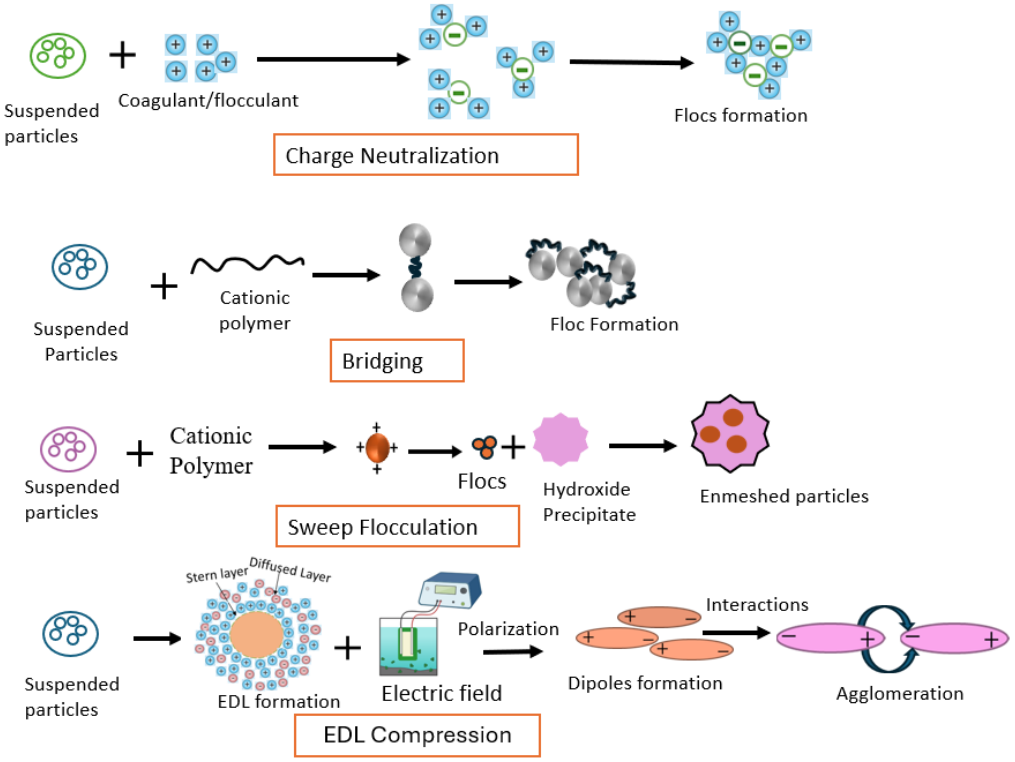

Figure 1.4. Different mechanisms of coagulation/ flocculation in water and wastewater treatment.

1.5. Fundamentals of applied surface chemistry

EDL formation and electrokinetic

Electric double layers spontaneously form when surfaces with ionizable groups are suspended in polar solvents like water. Water's high dielectric constant facilitates surface group dissociation, leading to the surface acquiring a net charge per unit area. This imbalance prompts the release of oppositely charged counterions into the solvent, often supplemented by microscopic ions. The double layer forms as a result, balancing the surface charge by accumulating an opposite charge density, effectively screening its electrostatic potential (Hansen & Löwen, 2000).

The dense inner layer, referred to as the "Stern layer," is primarily composed of counterions. Moving beyond the Stern layer, the electrostatic effects resulting from the surface charge decrease in accordance with Debye's law (Fig. 1.5). This law states that the strength of the field reduces by a factor of 1/e with each Debye length. Theoretically, this electrostatic influence extends infinitely, but experimental observations demonstrate its presence only within a few nanometers of the particle surface (Bhattacharjee, 2016). Charged nanoparticles generate an electrostatic field, resulting in the development of a diffuse layer containing both counterions and co-ions, extending past the Stern layer. Together with the Stern layer, this diffuse layer constitutes the EDL (McNamee, 2019). The composition of the diffuse layer is dynamic and varies depending on factors such as pH, ionic strength, and concentration. Within this diffuse layer lies a hypothetical plane serving as the interface between the moving particles and the surrounding dispersant layer during electrophoresis. This plane is termed the slipping or shear plane, and the zeta potential (ZP) represents the potential at this interface between particles and fluid. The Greek letter ζ (zeta) was originally used in mathematical equations to describe this phenomenon, hence the name "zeta potential." The potential on the particle surface itself is referred to as the Nernst potential (ψ^0) and cannot be directly measured. As particles move away from the surface in the dispersion, the electrostatic field weakens (Eq. 1.1) (Sennett & Olivier, 1965).

$$\psi = \psi_d e^{-kx} \tag{1.1}$$

Here, ψ is surface potential, x is the distance from the stern layer, ψ_d is surface potential at the stern layer, and κ is the Debye length. In case when the slipping plane is close to the Stern layer, then the $\psi_d \approx \zeta$ and therefore eq.2 will be modified as eq (1.2):

$$\psi = \zeta \mathrm{e}^{-kx} \tag{1.2}$$

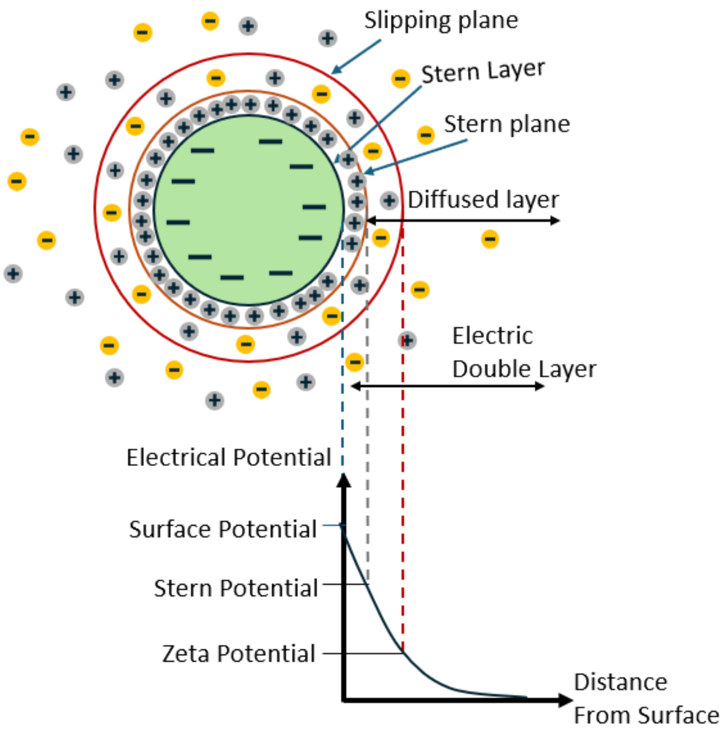

Figure 1.5. Illustration of electric double layer on the surface of colloidal particle. In the energy potential curve, the surface potential is the electrical potential surrounding the particle while the stern potential is the electrical potential at the Stern layer, and the zeta potential is the electrical potential at the slipping plane.

Surface charge and zeta potential

Zeta Potential (ZP), also known as electrokinetic potential, characterizes the potential at the slipping/shear plane of a colloidal particle when subjected to an electric field. It signifies the work needed to transport a unit positive charge from infinity to the particle's surface without acceleration, reflecting the potential difference between the EDL of electrophoretically mobile particles and the surrounding dispersant layer. ZP serves as a critical factor in understanding colloidal systems, acting as a pivotal stability indicator for colloidal dispersions. It regulates the magnitude of electrostatic repulsion between particles and determines their aggregation or dispersion (Kaszuba, Corbett, Watson, & Jones, 2010). Another significant application of ZP is in evaluating the surface charge of colloidal particles, determined by the direction of particle movement during electrophoresis. However, it's important to note that ZP doesn't directly measure charge or charge density; rather, it assesses surface potential. Therefore, only the magnitude of ZP holds significance, while the positive/negative polarity shouldn't be equated with surface charge or charge density. Zeta potential measurements provide valuable insights into colloidal behavior, with various factors capable of altering ZP from positive to negative and vice versa under differing conditions such as pH, temperature, or electrolyte concentration (Uskoković et al., 2009).

Surface interactions and colloidal particle aggregation

The overall interaction between two surfaces is relatively insensitive to changes in electrolyte concentrations, unlike EDL interactions. The van der Waals interaction (Anderson, Donaldson, Zeng, & Israelachvili, 2010; Israelachvili, 2011) is represented in eq.1.3

$$W \propto -\frac{1}{D^n} \qquad (1.3)$$

There are two distinct scenarios to consider: (1) For highly charged surfaces in dilute electrolytes, interactions are significantly influenced by both forces. These interactions tend to be repulsive when the surface charge density is high and the electrolyte concentration is low, resulting in a greater Debye length. Repulsion typically occurs within a range of 1 to 5 nm, forming a substantial energy barrier. In concentrated electrolytes, however, a secondary minimum typically exists beyond 3 nm before the energy barrier converges (Smith, Borkovec, & Trefalt, 2020). (2) In colloidal systems, when surface charge density is low, the energy barrier is reduced, leading to the aggregation of colloidal particles, known as flocculation. At high electrolyte concentrations and below a certain charge potential, the energy barriers fall below zero (Eq. (1.1), causing flocculation to increase rapidly (Song Li et al., 2023). As the surface charge approaches zero, the two surfaces attract each other strongly via Van der Waals forces. Lowering the pH, also increasing cationic binding, or enhancing the screening of double-layer repulsion by elevating ionic concentrations contribute to lowering surface charge and promoting colloidal aggregation (Israelachvili, 2011).

1.6. DC-initiated flocculation of algae (Scenedesmus dimorphus)

Microalgae are recognized as promising source material for producing biofuels and value-added products such as pharmaceuticals and cosmetics because of their high yield and efficient photosynthesis. However, challenges persist in harvesting microalgal biomass from suspended culture systems. These difficulties arise from factors, such as the small size of algal cells, their strong affinity for water, low density, and negative cell surface charge. The expensive process of harvesting microalgal biomass poses a major obstacle, accounting for a significant portion of overall production costs (G. Muhammad et al., 2021). Another challenge lies in foreign materials introduced during microalgal harvesting, e.g., chemical flocculants for enhanced gravitational or mechanical separation. These challenges impede the feasibility of large-scale production as a renewable feedstock material (Fayad, Yehya, Audonnet, & Vial, 2017). To mitigate these challenges, there is a need for a cost-effective and contamination-free harvesting method.

Electro-flocculation (EF) is gaining interest as a promising alternative to traditional coagulation/flocculation methods, which rely on chemical coagulants like metal salts and polymers. This approach introduces unique chemical and physical conditions within the system: (1) Reactive electrodes generate active coagulant species via electrolytic oxidation of the anode material, leading to precipitation reactions with hydroxyl species from the cathode and the formation of metal (oxy)hydroxides, promoting flocculation (Harif & Adin, 2007). Moreover, (2) the release of gases during electrolysis aids in pushing minute flocs to the surface, facilitating flotation. (3) Inert electrodes such as titanium do not produce metal ions for coagulation but polarize the electric double layer (EDL) of particles, forming dipoles within the diffused layer that promote flocculation through interactions (Shih, Molina, & Yamamoto, 2018). Research into EF has expanded significantly over the past decade, with studies exploring its applications in various fields, particularly in colloidal and organic matter removal. Both reactive and non-reactive anodes contribute to facilitating the flocculation process through different mechanisms.

In DC-initiated flocculation of microalgae the application of a DC voltage across two parallel plate inert electrodes to polarize (deform) the electric double layer (EDL) surrounding suspended microalgal cells. The polarization leads to the formation of electric dipoles and therefore induces dipole interactions, including both attraction and repulsion (Fig. 1.5) (Shih, Molina, & Yamamoto, 2015). The electrostatic attractive forces facilitate the collision and subsequent coalescence of microalgal cells, thereby initiating the flocculation process. A similar approach has been well-established for destabilizing colloidal suspensions (Dobnikar, Snezhko, & Yethiraj, 2013). In addition to induced dipole attractions, the electrohydrodynamic convection of suspended particles in a DC field may also play a role in particle assembly (Dommersnes et al., 2013), potentially enhancing flocculation efficiency. For water treatment, DC-initiated flocculation was first introduced by a group of researchers in Japan (Mori, Nagashima, Ito, Era, & Tsubaki, 2019).

1.7. Influential factors for coagulation/flocculation processes

Dosage level

Understanding the influential factors that govern coagulation and flocculation processes is essential for optimizing water treatment efficiency and achieving desired treatment outcomes. The type and dosage of flocculants play an important role in discussing influential factors of flocculation and determining its efficiency (Shuangxi Li et al., 2020). Factors such as the charge density, molecular weight, and particle size of the flocculant molecules influence their ability to aggregate suspended particles and promote settling. Furthermore, the dosage optimization of flocculants is crucial to achieving the desired level of particle agglomeration without overdosing, which can lead to increased costs and potential negative impacts on water quality (Grenda, Gamelas, Arnold, Cayre, & Rasteiro, 2020; Lee et al., 2014).

Treatment time

Another influential factor is the treatment time, adequate treatment time is essential to allow for the formation of flocs and their subsequent growth before settling or filtration. Insufficient contact time may result in incomplete flocculation and poor water clarification, while excessive contact time can lead to unnecessary energy consumption and process inefficiencies (Amuda & Alade, 2006).

pH

The final pH of water and wastewater treatment significantly impacts flocculation performance. pH levels influence the charge properties of suspended particles and flocculant molecules, affecting their interactions and overall efficiency. Optimal pH conditions vary based on the flocculant type and water characteristics that emphasize the need for pH adjustment in flocculant process optimization (Saritha et al., 2017). Controlling these factors is essential for efficient flocculation and high-quality water treatment, necessitating systematic testing, parameter optimization, and continuous monitoring.

External electric field

When suspended particles in a liquid possess a negative charge, they form an EDL due to electrostatic forces, resulting in a high zeta potential. This zeta potential acts as a barrier preventing particle aggregation. Overcoming this barrier and promoting particle aggregation requires an external electric field stronger than the existing electrostatic forces to polarize the EDL (Shih et al., 2018). This polarization induces the formation of dipoles, aligning the particles effectively. Consequently, these aligned dipoles interact, leading to particle attraction and eventual aggregation.

1.8. Various flocculants for water and wastewater treatment in this dissertation

Cationic starch.

In Chapter 2 of this dissertation, the focus is on the development of bio-inspired materials derived from agricultural byproducts, such as potato peel waste (PPW), to enhance agricultural sustainability and foster a circular economy. Specifically, starch extracted from PPW is transformed into cationic starch (CS) through a simple wet chemistry process. The resulting cationic starch exhibits promising capabilities in efficiently removing suspended solids (SS) from swine and dairy wastewater, with notable SS removal efficiencies of 72 mg TSS/mg flocculant for swine wastewater and 162.9 mg TSS/mg flocculant for dairy wastewater. Moreover, significant reductions in SS levels, reaching 27.9% for swine wastewater and 58% for dairy wastewater, are observed, indicating the potential of these bio-inspired materials for wastewater treatment applications. Further improvements in SS removal are anticipated through the enhancement of the degree of substitution (DS) of cationic starch using various catalysts or solvents.

Cationic cellulose

In Chapter 3, a novel approach was taken to enhance the functionality of commercial cellulose by transforming it into cationic cellulose (CC) through a homogeneous etherification process with 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) in an alkaline environment. By adjusting various reaction parameters, CC samples with different degrees of substitution (DS) were synthesized, with optimal conditions identified: a 4-hour reaction duration encompassing alkalization and etherification, utilizing 0.124M CHPTAC, 3 M NaOH, and maintaining a reaction temperature of 60°C. Structural analyses through FT-IR, DSC, SEM, and NMR techniques confirmed the successful incorporation of quaternary ammonium groups into the cellulose backbone. A CC sample with a DS value of 0.845 was assessed for its effectiveness as a flocculant/sorbent across three distinct application scenarios. Encouraging results were obtained, demonstrating substantial efficiency in the capture and removal of algal biomass (up to 93.0%), total suspended solids (TSS) (up to 53.3%), and model organic (MO) compounds (up to 53.0%) from microalgal cultural broth, dairy wastewater, and synthetic dye wastewater, respectively.

DC-initiated flocculation

Chapter 4 of the dissertation demonstrates how direct current (DC)--initiated flocculation can be a simple yet effective method for harvesting microalgae. The research methodically investigates how varying DC voltage and energizing time impacts the flocculation of Scenedesmus dimorphus suspensions. Results show that under optimal conditions (DC=20V and t=60 min), a high flocculation efficiency of 94% is achieved. Although slightly less efficient compared to traditional methods such as chemical flocculation with Alum or electrocoagulation using Al electrodes, DC-initiated flocculation produces microalgal biomass with significantly lower aluminum content. The mechanism behind flocculation in a DC electric field is attributed to the polarization of the electrical double layer (EDL) of microalgal cells, leading to induced dipole interactions.

Moving forward

Moving forward, our work aims to advance the application of innovative technologies for flocculating suspended particles in both water and wastewater. Drawing inspiration from natural occurrences, and the structural and electrochemical features of natural materials, we seek to create natural flocculant platforms. Based on the findings presented in Chapter 3 and Chapter 4, we will further explore the development of novel flocculants using various techniques. Furthermore, dipole formation by polarizing the EDL of the suspended particles using an external electric field will function similarly to chemical flocculants, effectively neutralizing opposite charges to improve flocculation efficiency. By pursuing research in this direction, we strive to contribute to the advancement of sustainable and efficient methods for water and wastewater treatment. Ultimately, our goal is to address environmental challenges and ensure universal access to clean water resources. By undertaking this research path, our goal is to make substantial contributions to the progress of sustainable and effective techniques for water and wastewater treatment. Ultimately, our primary aim is to tackle environmental challenges and guarantee fair access to clean water resources for everyone.

CHAPTER 2

Flocculation of livestock wastewater using cationic starch prepared from potato peels

The research presented in Chapter 2 has been published in *Environmental Science: Water Research & Technology*

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Abstract:

A cationic starch flocculant was prepared from potato peel waste (PPW) for enhanced solid-liquid separation of livestock wastewater, thereby promoting the agricultural circular economy. The starch extracted from PPW was cationized by incorporating a cationic moiety into the backbone of starch via etherification with (3-chloro-2 hydroxypropyl) trimethylammonium chloride (CHPTAC). Using response surface methodology, a water-soluble cationic starch sample with a degree of substitution (DS)=0.86 was obtained with 1 wt% starch, 0.124 M CHPTAC, 2.0 M NaOH, isopropanol solvent, 60ºC reaction temperature, and 5-h reaction time. Lab characterization confirmed the successful cationization of starch. The DS=0.86 sample was further studied for its flocculation performance. Total suspended solids (TSS) were reduced by 22-28% and 35-58% for prescreened swine and dairy wastewater samples, respectively. The TSS removal per unit dosage of flocculant was greater for the cationic starch than many commercial flocculants. This study is expected to stimulate the valorization of agricultural byproducts.

Keywords: flocculation, livestock wastewater, solid separation, cationic starch, waste valorization

2.1. Introduction

Potato (*Solanum Tuberosum*) is the fourth most consumed crop worldwide, with an annual production of ~370.43 million metric tons in 2019 (Djaman, Irmak, Koudahe, & Allen, 2021; Waste ManagementStat, 2012). Other than directly cooked, over 50% of harvested potatoes are processed into food products, food ingredients, animal feed, and starch. Massive amounts of potato peel waste (PPW) are produced from these processes as a byproduct: 70×10^3-140×10^3 tons per year accounting for 15-40 % of the mass of primary processing products (Sepelev & Galoburda, 2015). Most of the produced PPW is disposed of in landfills as solid waste, adding zero value (if not additional cost) to the agricultural economy. Eco-friendly utilization of PPW has been pursued, including food preservatives, pharmaceutical ingredients, animal feed, and feedstock for bioenergy production (Gebrechristos & Chen, 2018). Many of these applications are tied to the unique composition of PPW.

PPW is rich in polysaccharides and phenolic compounds (e.g., lignin). Low-molecular-weight phenolic compounds such as garlic acids and flavonoids have been tested as potential antioxidants or antimicrobials for food preservation (Sampaio et al., 2020). Starch is the single most abundant component, accounting for up to 52% of dry potato peel mass (Arapoglou, Varzakas, Vlyssides, & Israilides, 2010). Other abundant components include non-starch polysaccharides, lignin, and protein (Camire, Violette, Dougherty, & McLaughlin, 1997). No composition information on potato peel starch is available. Assuming the same composition as potato starch, it would contain 73-79 % amylopectin and 12-27 % amylose (Hongcai Zhang, He, Wang, & Chen, 2021). Although starch can be utilized for various purposes such as ethanol fermentation (C.-W. Lin, Tran, Lai, I, & Wu, 2010), novel material synthesis (Hussein et al., 2021), and enzyme production (dos Santos, Gomes, Bonomo, &

Franco, 2012), an industrial-scale application is hindered by the starch's poor water insolubility, easy retrogradation after gelatinization, and high gelatinization temperature (Ge et al., 2021).

A potential application of PPW is to produce cationic starch flocculants for wastewater treatment. This involves the chemical modification of starch granules extracted from PPW. Various chemical methods (e.g., esterification, etherification, oxidation, carbonization, and crosslinking with other polymers) are available for starch modification, aiming to substitute hydroxyl with desired functional groups at typically C-2, 3, and/or 6 in starch backbones (Fan & Picchioni, 2020). Starch can be cationized through etherification reactions with tertiary amino and quaternary ammonium reagents under homogeneous reaction conditions. The tertiary amino and quaternary ammonium groups at C-2, 3, and/or 6 grant positive charges (cations) to the modified starch (Lappalainen et al., 2017; Liu et al., 2022). The commonly used cationic reagents included glycidyl trimethyl ammonium chloride (GTAC) and 3-chloro-2 hydroxypropyl trimethylammonium chloride (CHPTAC) (Liu et al., 2022). Both could lead to a high degree of substitution (DS) in prepared cationic starch. Homogeneous alkaline catalysts such as NaOH, $NH_4(OH)$, and $Ca(OH)_2$ were required for starch cationization (Almonaityte, Bendoraitiene, Babelyte, Rosliuk, & Rutkaite, 2020). The addition of alcohols in an alkaline aqueous solution was found to increase the DS of cationic starch. However, no systematic study of the preparation conditions was performed (W. Li et al., 2021). An optimal preparation condition can be achieved by examining individual preparation parameters and their interactions via improved experimental designs such as response surface methodology (RSM) (N. Haleem et al., 2021).

Livestock farming generates a large amount of wastewater and it, along with poultry farming, constitutes the third largest source of water pollution (after industrial and domestic sources) (T. Zhang, Gao, Wang, Liu, & Jiang, 2019). Among various treatment methods, solid separation is believed by many to be the most cost-effective method for phosphorus management (Burton, 2007; Hjorth, Christensen, Christensen, & Sommer, 2011). This is because most of the phosphorus in livestock wastewater occurs as solid particles rather than in dissolved forms. The suspended solids can be removed with gravitational settling, mechanical, and chemical methods. An example of the chemical method is adding flocculants/coagulants to enlarge the particle size, thereby enhancing the solid separation effectiveness of gravitational and mechanical (e.g., rotary drum, centrifuge, and roller press) processes (Garcia, Szogi, Vanotti, Chastain, & Millner, 2009). Besides phosphorus management, other benefits of solid separation include reduced odor nuisance (H. Zhang & W. Westerman, 1997) and improved suitability of livestock wastewater for irrigation and anaerobic digestion (Burton, 2007).

The Upper Midwest (Iowa, Michigan, Minnesota, Wisconsin, North Dakota, and South Dakota) is a livestock production hub in the U.S. For example, with a population of 0.88 million, South Dakota ranks sixth in cattle and tenth in the largest swine-producing state. The region is also known for its crop production. To address growing livestock wastewater management challenges, we propose a “from agriculture, back to agriculture” circular economy approach, that is, treating livestock wastewater with chemicals prepared from agricultural byproducts. Specifically, in this study, cationic starch – a natural flocculant prepared from PPW – was tested for solid removal from livestock wastewater. The research objectives were to (1) prepare cationic starch from PPW and optimize its preparation conditions with RSM; (2)

characterize the prepared cationic starch flocculants; and (3) examine the effectiveness of the flocculants in solid removal from swine and livestock wastewater.

2.2. Methods and Materials

2.2.1. Starch extraction from potato peels

Around 1.8 kg of brown skin potatoes acquired from a local supermarket were manually peeled after rigorous washing with hot water. The peels (~176 g) were chopped into small pieces and further ground into fine granules using a coffee grinder. Deionized water was then added to the granules to create a slurry. After homogenization for 30 min, the slurry was sieved using a 135-mesh polyester screen, and the acquired filtrate was centrifuged at 3000 rpm for 12 min. Pellets (wet starch) in the bottom of centrifuge tubes were collected and dried at room temperature for 5 hrs. The starch was ground into fine powders and stored at ~4ºC in a lab refrigerator.

2.2.2. Preparation of cationic starch

The preparation adopted a protocol revised by (Chittapun, Jangyubol, Charoenrat, Piyapittayanun, & Kasemwong, 2020). It involved two steps: alkalization and etherification (Fig. 2.1). During alkalization, 2 g of the extracted starch was mixed with 200 mL isopropanol and the mixture was stirred at 300 rpm for 15 min. Ten milliliters of a NaOH solution (0.5, 1.0, 1.5, 2, or 2.5 M; depending on experimental design) was then added dropwise to the mixture while stirred, and the stirring continued for another 30 min. During etherification, 3-7 mL (i.e., 0.053-0.124 M; depending on the design) of 60% 3-chloro-2-hydroxy propyl trimethyl ammonium chloride (CHPTA) was added to the alkalized starch at an elevated temperature (~60ºC) and the heated stirring continued for 2-5 hrs. After cooling down to room temperature, the product was added with 99% ethanol (to stop the etherification reaction) and neutralized to pH=7 using 0.1 M HCl. Upon vacuum-filtration, the acquired starch product was washed 3-5 times with 99% ethanol to remove unreacted reagents and byproducts and air-dried overnight in a lab oven at 40°C. All the chemical reagents were purchased from Sigma Aldrich and of analytical grade.

2.2.3. Degree of substitution (DS) determination

For cationic starch, DS is defined as the average number of hydroxyl groups substituted by quaternary ammonium groups at C-2, 3, and 6 in its backbone (Abdul Hadi, Wiege, Stabenau, Marefati, & Rayner, 2020). It was calculated as (You et al., 2022):

$$\mathrm{DS} = \frac{162.15 \times N\%}{14.01 \times 100 - CR \times N\%} \tag{2.1}$$

Where CR is the molecular weight of cationic reagent, N% refers to the content of total nitrogen in a cationic starch sample and it was determined using a TNT plus 826 test kit (Hach Method 10208). For accurate determination of N%, five replicates were analyzed per sample (Nasir, Abdulmalek, & Zainuddin, 2020) and a small standard deviation (<0.5%) was achieved.

2.2.4. Optimization of preparation conditions

Design Expert 11.0 (Stat-Ease Inc., Minneapolis, MN, USA) was used for experimental design and associated statistical analysis. Three independent variables (preparation parameters) were examined: reaction time, CHPTA concentration, and NaOH concentration; with DS serving as the response. The experimental design followed central composite design (CCD), a prevalent response surface methodology (RSM) method. Twenty experimental conditions were investigated (Table 2.1). Each ran in triplicate to calculate an average DS value. The acquired data were analyzed with ANOVA and further fitted with a polynomial regression equation (Equation 2), following a procedure described by Boulifi et al (Boulifi, Aracil, & Martínez, 2010).

Table 2.1. Experimental design for optimizing cation starch preparation conditions.

Experiment No.	**Factor 1** A: NaOH concentration (mol/L)	**Factor 2** B: Cationic reagent (mL)	**Factor 3** C: Reaction time (min)	**Response 1** Degree of substitution (DS)
1	1.5	5	4	0.57
2	2.5	7	2	0.55
3	0.5	5	2	0.17
4	2	4	2	0.74
5	2.5	3	5	0.54
6	1	6	5	0.49
7	1.5	5	4	0.57
8	0.5	5	2	0.17
9	1	6	3	0.43
10	0.5	3	4	0.12
11	1.5	5	4	0.57
12	0.5	7	4	0.19
13	1	3	3	0.39
14	1.5	7	2	0.58
15	2.5	7	4	0.64
16	1.5	5	4	0.57
17	2	4	2	0.74
18	0.5	4	5	0.15
19	2	7	5	0.86
20	2.5	6	3	0.60

2.2.5. Flocculation experiments

Raw swine wastewater samples were acquired from a wean-to-finish barn managed through the Swine Education & Research Facility of South Dakota State University (Flandreau, SD, USA). The barn houses ~1200 head of hogs and uses a

2.4-m deep pit under slatted concrete floors for waste collection and storage. Raw dairy wastewater samples were acquired from a 2000-head dairy farm near Lake Benton, MN, USA. The farm uses a sand-bedded free-stall system and sand lanes to separate sand from wastewater before feeding it to an anaerobic digester. The wastewater was collected after sand separation. The acquired wastewater samples were prescreened with a 35-mesh sieve to large solid particles and then kept in a refrigerator at 4 ºC before flocculation experiments. No natural solid settling or stratification was observed. The wastewater samples remained vertically homogeneous after one month's storage.

The flocculation experiments (i.e., jar testing) were performed on a six-paddle jar tester (Phipps & Bird Model 7790-901B; Fisher Scientific Inc., Richmond, VA, USA). A standard protocol described by (Yamamura et al., 2020) was followed during the experiments. First, 1 g of cationic starch was stir-mixed with 100 mL of deionized water for 30-40 min to create a clear colloidal solution. Then, the prepared flocculant solution was added to 0.5-L swine and dairy wastewater to approach target dose levels (80, 125, 150, 200, and 250 mg/L). Three jar test replicates were carried out at each dose level. Before the addition of flocculants, the pH of swine wastewater in each jar testing beaker was adjusted to pH=8 using a diluted HCl solution, while no pH adjustment of dairy wastewater was conducted. The flocculant solution was added to all six beakers simultaneously. To stimulate coagulation, the wastewater samples were stirred intensively at 300 rpm for 1.0 min, then gently at 20 rpm for 20 min to promote flock formation. The mixtures were allowed to sit for 24 h. for the flocks to settle down. In beakers with cationic starch added, stratification of swine and dairy wastewater was observed, with solids settling in the bottom of each beaker. In beakers with cationic starch added (controlled samples), no stratification of swine and dairy wastewater was seen. Liquid samples (5 mL) were taken from each beaker at ~2 cm depth from the top. The total suspended solids (TSS) of the liquid samples and control samples (i.e., wastewater without flocculants added) were measured using glass fiber filters (Whatman GF/A, with 1.6-µm pore size) based on the classic gravimetric method (Philip A. Hansel, Guy Riefler, & Stuart, 2014).

2.2.6. Characterization of cationic starch

Morphological analysis was done with a Hitachi S-3400N scanning electron microscope (SEM) (Hitachi Ltd., Tokyo, Japan) at an accelerating voltage of 10.0 kV. A ~1.0-mg starch or cationic starch sample was loaded onto a two-sided adhesive carbon tape and coated with a thin gold layer. Images were taken at multiple magnifications to examine the size and shape of starch granules.

IR spectra were measured with a Perkin-Elmer Spectrum 100 FT-IR spectrometer (PerkinElmer Inc., Waltham, MA, USA). A powdered sample was mixed with KBr (2:98 w/w) and pressed into a transparent disk. A transmittance spectrum was derived from 28 scans of the sample at a resolution of 4 cm^{-1}. The purpose of FT-IR analysis was to identify functional groups indicative of successful starch modification.

DSC analysis was conducted on a PerkinElmer DSC 6000 analyzer (PerkinElmer Inc., Waltham, MA). A ~10-mg sample was loaded in a platinum sample pan and purged with N_2 at a flow rate of 20 mL/min. After initial baseline determination, the sample was heated from 20 ºC to 450 ºC at a ramp rate of 10 ºC/min. An empty pan was also subject to DSC analysis and used as a reference for signal subtraction. The purpose of the analysis was to compare original starch versus cationic starch samples in their glass transition (T_g) and decomposition temperatures.

A shift in the temperatures indicates a successful modification (El-Naggar, Abdel-Aty, Wassel, Elaraby, & Mohamed, 2021).

Like FT-IR, NMR was used to determine the functional groups indicative of cationic starch. The analysis (^{1}H NMR) was conducted on a Bruker Avance 300 MHz NMR spectrometer (Bruker Corp., Billerica, MA). Tetramethyl silane was selected as an internal standard. Samples were dissolved in dimethyl sulfoxide-d6 (DMSO-d6) Chemical shifts were recorded with MestReC software and expressed in ppm.

2.3. Results and Discussion

2.3.1. Optimization of preparation conditions

Three experimental parameters were examined: NaOH concentrations, CHPTA concentrations, and reaction time. DS characterizes the average number of substituted ammonium groups per anhydro glucose unit (AGU) Thus, it was selected as the benchmark measure of the quality of prepared cationic starch. As aforementioned, 20 preparation conditions following the CCD design (Table 2.1) were studied to determine the optimal preparation condition (Fig. 2.1). A similar method was reported by Hivechi et al. (Hivechi, Bahrami, Arami, & Karimi, 2015) for optimizing DS. Through the experiments, a second-order polynomial equation was obtained (Eq. 2.2).

$$\begin{aligned}\mathrm{DS} = 0.670914 + 0.883388A - 0.138120B - 0.357549C \\ + 0.000892AB - 0.000661AC + 0.031826BC \\ - 0.218739A^2 + 0.003564B^2 + 0.029004C^2\end{aligned} \tag{2.2}$$

where, A, B, and C correspond to NaOH concentrations, the volume of the cationic reagent added, and the reaction time, respectively.

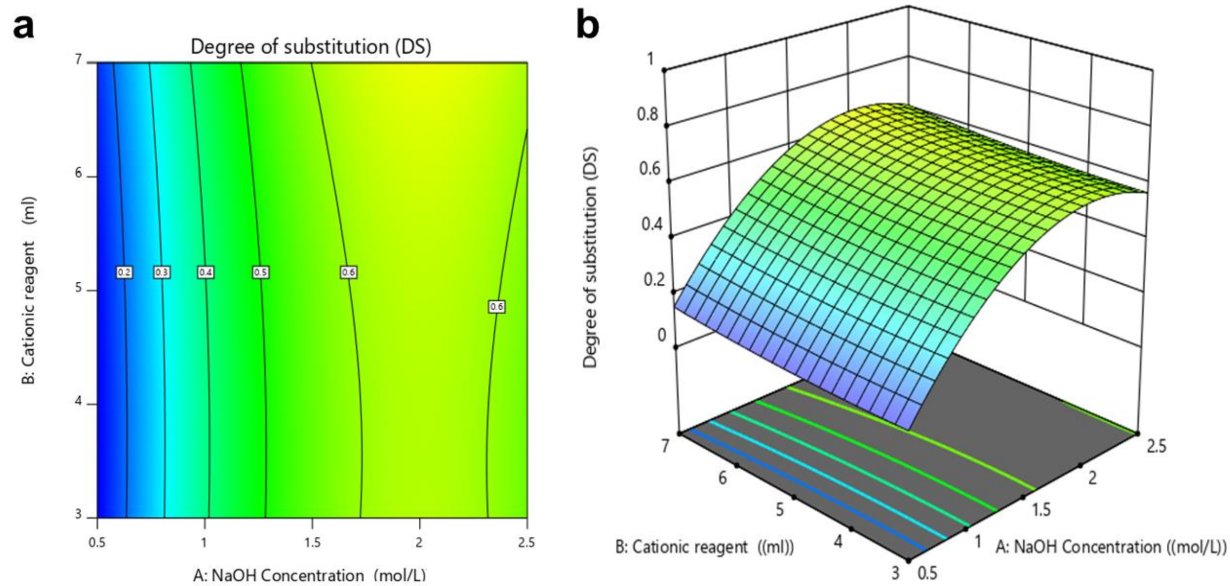


Figure. 2.1. Response surface plots showing the co-effects of NaOH concentration, volume of cationic reagent, and reaction time on the DS of cationic starch.

The analysis of variance (ANOVA) test was conducted to further investigate the effect of each item (e.g., A and AB) in Eq. 2.2 (Table 2.2). Four significant influential items were identified: A, BC, A^2, and C^2, with $p<0.05$. The same threshold ($p<0.05$) was commonly used in RSM-CCD analysis to determine the significance of correlation (R. Lin et al., 2014). The most significant factor was found to be the NaOH concentration. As a measure of the goodness-of-fit, the adjusted determination of the coefficient was calculated (Adj $R^2 = 0.9827$), along with the predicted determination of the coefficient (Pred $R^2 = 0.8349$). The high R^2 values and a low coefficient of variation (CV = 5.92%) indicate that the experiments were precise and reliable (Yetilmezsoy, Demirel, & Vanderbei, 2009). A comparison of the model-predicted versus actual DS was shown in Fig. 2.2 which again affirms the good-fitting performance of Eq. 2.2.

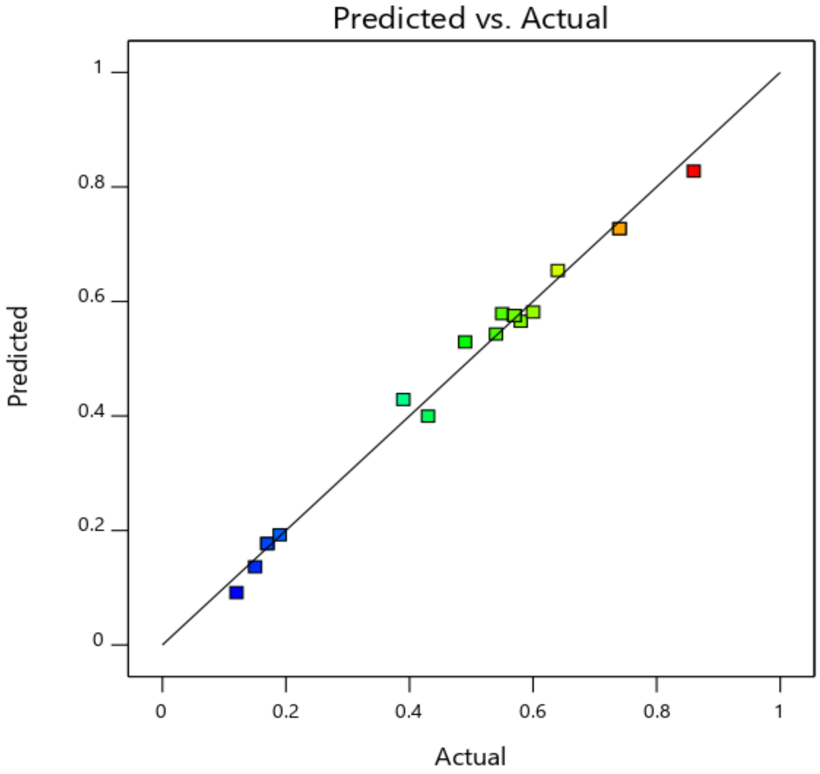


Figure 2.2. Diagnostic plot of the quadratic model showing predicted responses versus actual responses.

Table 2.2. ANOVA for a quadratic model for degree of substitution (DS) as a response of experimental variables.

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value** [a]
Model	0.8878	9	0.0986	121.01	< 0.0001
A-NaOH concentration	0.4739	1	0.4739	581.43	< 0.0001
B-Cationic reagent	0.0035	1	0.0035	4.35	0.0636
C-Reaction time	0.0003	1	0.0003	0.3538	0.5652
AB	0.0000	1	0.0000	0.0189	0.8933
AC	5.74E-06	1	5.74E-06	0.0070	0.9348
BC	0.0425	1	0.0425	52.11	< 0.0001
A^2	0.1385	1	0.1385	169.96	< 0.0001
B^2	0.0006	1	0.0006	0.7345	0.4115
C^2	0.0147	1	0.0147	17.99	0.0017
Residual	0.0082	10	0.0008		
Lack of Fit	0.0082	5	0.0016		
Pure Error	0.0000	5	0.0000		
Cor Total	0.8959	19			

The effect of NaOH concentrations was further examined since it was identified as a top influential factor. As shown in Fig. 2.3 (Note: a graph created by the Design Expert software), DS increased with the molar concentration of NaOH until it reached DS=0.86 at 2.0 M NaOH. A further increase in NaOH concentrations led to a decrease in DS. Cationization of starch is achieved through etherification reactions with cationic reagents, and etherification reactions generally prefer the presence of a strong base like NaOH to increase the nucleophilicity of hydroxyl groups in a starch backbone. Furthermore, the extra hydroxyl ions released from NaOH can shift the reaction equilibrium towards the product side. However, at high NaOH concentrations, a side reaction of cationic reagent with sodium hydroxide could occur, suppressing the cationization reactions (Sableviciene, Klimaviciute, Bendoraitiene, & Zemaitaitis, 2005). In short, an optimal NaOH concentration (2.0 M) existed for the etherification of extracted starch.

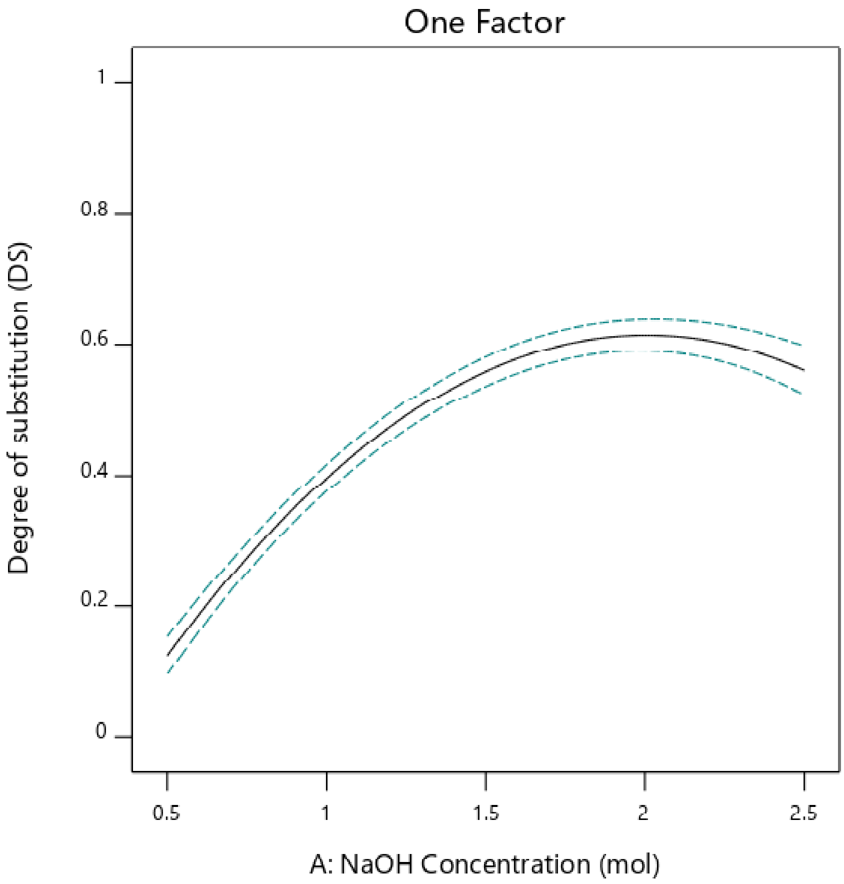


Figure 2.3. Effect of NaOH concentrations on the degree of substitution (DS) of cationic starch. Note: The figure was created by the Design Expert software. The solid black line shows the predicted DS values at the reaction time of 3.5 h (average of 2-5 h) and the CHPTAC concentration of 0.088 M (average of 0.053-0.124M), and the blue dash lines represent the 95% confidence interval of the predicted DS.

2.3.2. Characterization of cationic starch

Morphology

Fig 5 shows the SEM images of potato peel starch (DS=0) and cationic starch samples (DS=0.12 & DS=0.86). The unmodified starch (DS=0) largely consisted of oval granules with smooth surfaces and some curtailed sides. No obvious defects or impurities were observed (Fig. 2.4a). The same morphology was reported by Nasir and his coworkers. (Md Nasir, Abdulmalek, & Zainuddin, 2020). For cationic starch samples, unreacted cationic reagents and by-products were removed during the purification step. However, the removal might be incomplete, as indicated by NaCl crystals trapped on the surface of some cationic starch granules (Fig. 2.4c). A similar observation was made by Almonaityte et al. (Almonaityte et al., 2020). Defects were seen on cationic starch granules, especially for the DS=0.86 sample (Figs. 2.4b and c), likely due to the penetration of the cationic reagent into the starch granules. The cationization reaction was considered to disrupt the structure of starch, reduce the hydrogen bonding, and facilitate water uptake, thereby increasing the water solubility of cationic starch (Pi-xin et al., 2009).

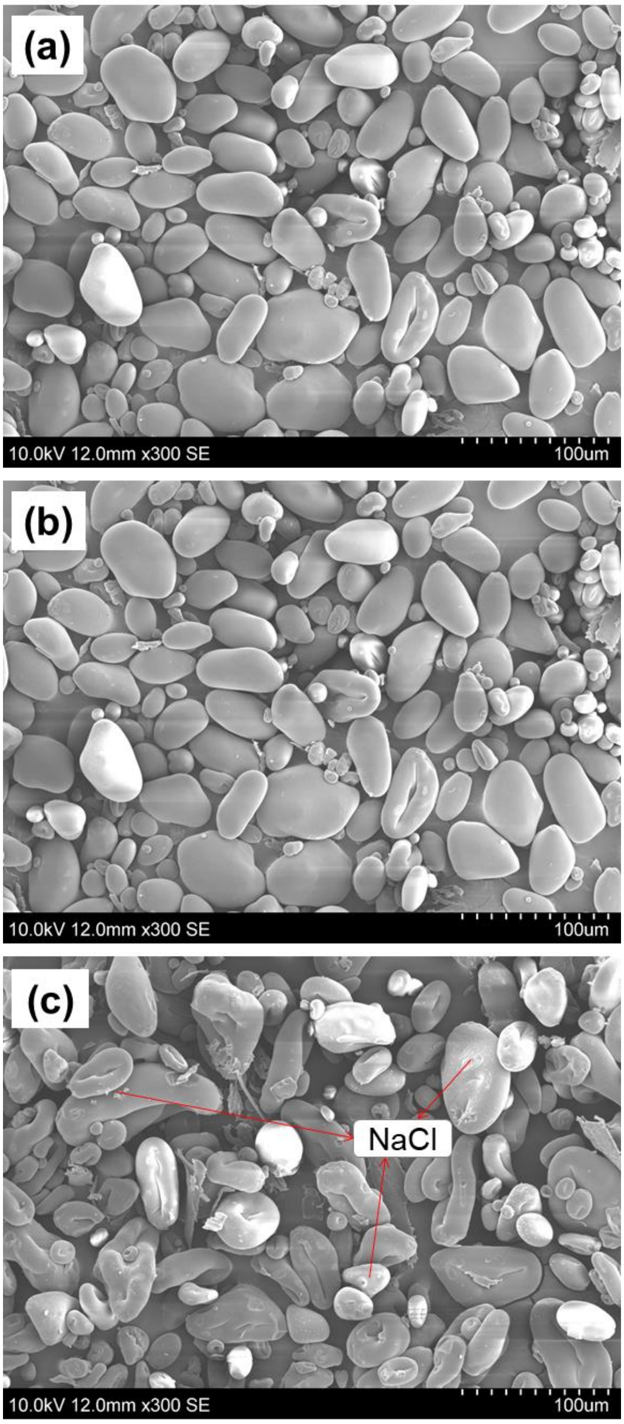


Figure. 2.4. Scanning electron microscope (SEM) images of (a) raw starch extracted from potato peels (DS=0), (b) cationic starch with DS=0.12, and (c) cationic starch with DS=0.86. All the images were taken at ×300 magnification.

FTIR spectra

FTIR analysis confirmed the successful cationization of potato peel starch (Fig. 2.5). Compared with the unmodified starch sample, a bathochromic shift of the peaks at 959 cm^{-1} and 1480 cm^{-1} was seen for the modified samples (DS=0.12 and DS=0.86). This peak can be assigned the stretching vibration of the C–N bond of quaternary ammonium groups and, thus, is an indication of the successful incorporation of trimethyl ammonium groups (Pi-xin et al., 2009). It is noteworthy that given the large backbone of starch and the theoretical limit of substitution (only 1/3 hydroxyl groups can be substituted), unmodified and modified starch samples showed similar absorption bands. For example, broad bands at 3399 and 2937 cm^{-1} were attributed to the O-H and C-H bond stretching vibrations, respectively (S. Pal, D. Mal, & R. Singh, 2005); and the peaks at 990, 1086, and 1157 cm^{-1} were the characteristic absorption bands of AGU (Y. Wei, Cheng, & Zheng, 2008).

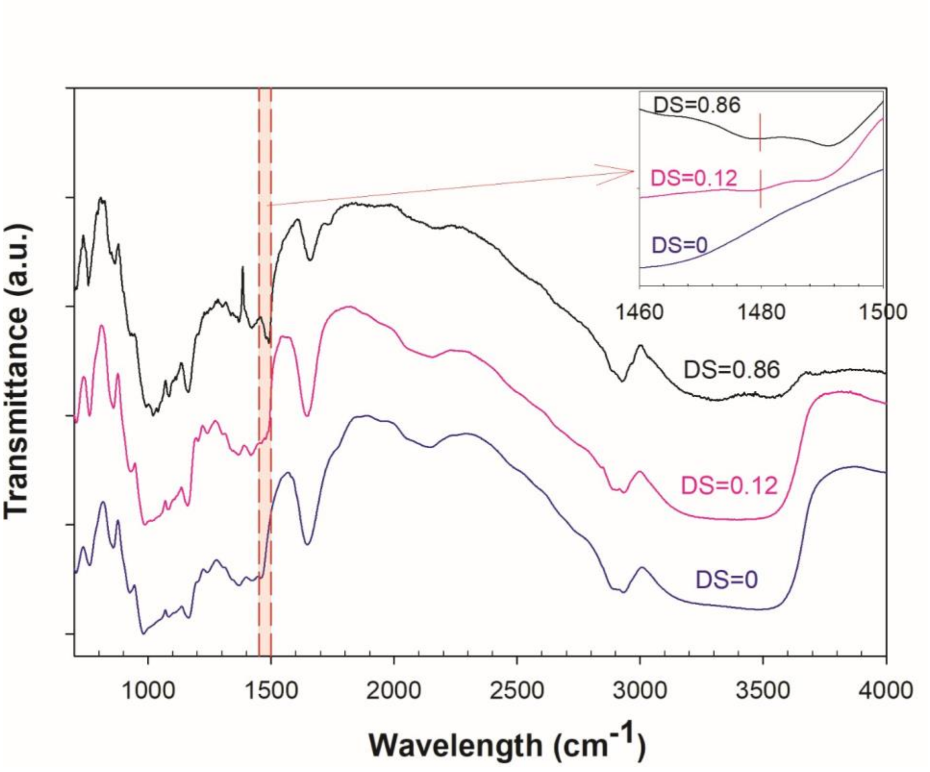


Figure 2.5. FTIR spectra (wave number 0-4000 cm^{-1}) for (a) raw starch extracted from potato peels (DS=0), (b) cationic starch with DS=0.12, and (c) cationic starch with DS=0.86.

NMR spectra

Another evidence of successful cationization is provided by NMR analysis. The NMR spectra of raw starch extracted from potato peels (DS=0) and cationic starch with DS=0.12, 0.86 are shown in Fig. 2.6. Due to the low solubilities of all samples, the spectra are dominated by the signals of trace water at 3.35 ppm and trace DMSO (mostly DMSO-d5) at 2.50 ppm even though the anhydrous DMSO-d6 was used and NMR sample preparation was done in a N2 glovebox (see Fig. 2.6a for the raw starch). Spectra in Fig. 2.6b-d are blown up and truncated to better show the signals from starch, and to better compare the intensities of peaks visually, the peak at 5.773 ppm, which is from the H on C-1, is set at the same height. Besides DMSO and moisture, THF and, to a lesser extent, EtOAc (with peaks at 4.106, 2.07, and 1.05 ppm), are also found in the spectra. These are common organic solvents present in the glovebox atmosphere. Nevertheless, NMR provides strong evidence of successful cationization. According to the literature (Wang & Xie, 2010), 1H NMR signal at ~3.1 ppm was ascribed to CH3–N+. This spectral region is shown in Fig. 5e-g for DS=0, 0.12 and 0.86 samples. Three new singlets at 3.125, 3.142, and 3.160 ppm are seen in Fig. 5f and 5g and can be assigned to the methyl groups in the ammonium salt covalently incorporated at three OH sites on C2, C3, and C6 of the glucose unit. An apparent doublet is found in the same spectral region of the raw starch, which is likely from an impurity in the starch and is also present in the spectra of the two cationic starch samples and partially overlaps with the 3.160 ppm peak of the ammonium. The peaks between 4.5 and 5.5 ppm were from three OH groups on C2, C3, and C6 of the glucose unit and impurities such as lignin. The intensity of OH peaks all decreased upon modification with CHPTAC. The region of 3.50-3.80 ppm, which is dominated by the trace THF, is where the peaks of the hydrogens on the glucose carbons (except C-1) are located. In the literature, Wang & Xie, 2010, the peaks between 4.5 and 5.5 ppm were mistakenly ascribed to the hydrogen atoms in the starch backbone (meaning the Hs on backbone carbon), and the peak at 3.6 ppm was mistakenly ascribed to O-H groups (Camire et al., 1997). It is noted that a small amount of lignin is present in the starch. No effort was made to remove it since it also contains OH groups and can be cationized as starch (Wang & Xie, 2010).

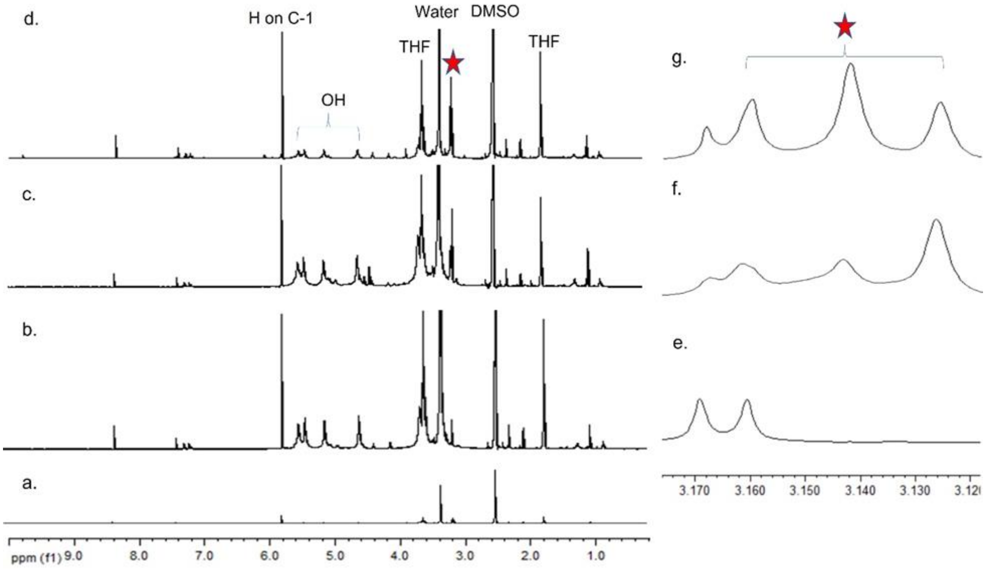


Figure 2.6. 1H NMR spectra of (a,b) raw starch extracted from potato peels (DS=0), (c) cationic starch with DS=0.12, (d) cationic starch with DS=0.86, (e,f,g) spectral region of 3.176-3.120ppm of DS=0, 0.12, 0.86, respectively.

DSC thermograms

DSC analysis results also supported the successful cationization of potato peel starch (Fig. 2.7). Firstly, the glass transition temperature (T_g) of the DS=0.86 sample (87.3ºC) was lower than that of the unmodified starch sample (96ºC). The decrease in T_g may be ascribed to the insertion of quaternary ammonium groups at C-2, 3, or 6 in the starch polymer backbone. Such insertion can increase the mobility of polymers due to reduced hydroxyl groups and accordingly reduced hydrogen bonds. The thermal degradation of the DS=0.86 sample started at 231°C, substantially lower than that of unmodified starch (281°C). A similar finding was reported by (Aggarwal & Dollimore, 1998; Wang & Xie, 2010).

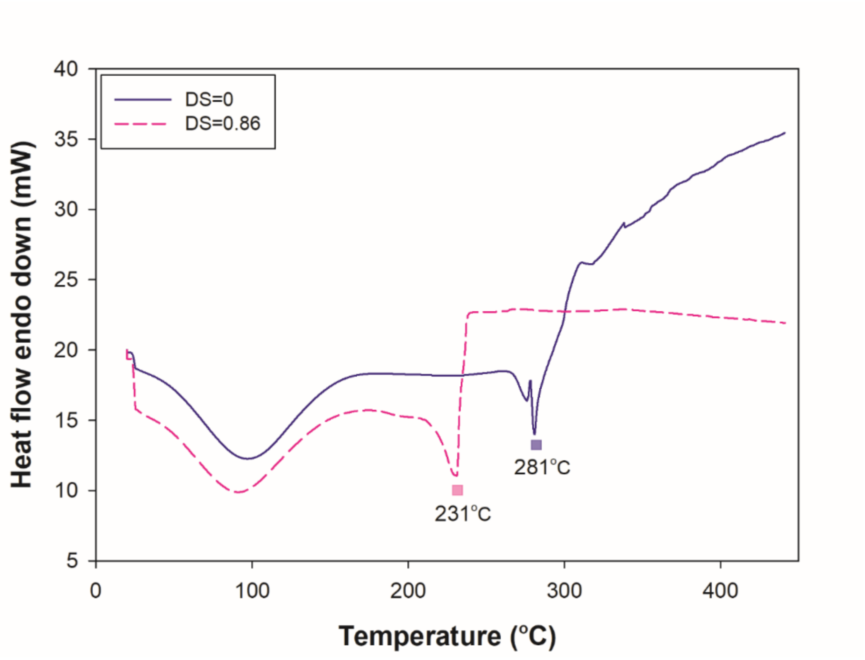


Figure 2.7. Differential scanning calorimetry (DSC) thermograms of (a) raw starch (DS=0) extracted from potato peels (blue line) and (b) cationic starch with DS=0.86 (red dashed line).

2.3.3. Flocculation performance

The solids in livestock wastewater can be classified as fiber (> 5 mm), coarse solids (1–5 mm), fine solids (20 µm–1 mm), colloidal particles (1–20 µm), and dissolved solids (< 1 µm) (Rolf, Weide, Brügging, & Wetter, 2021). In this study, the TSS contents of original swine and dairy wastewater samples were as high as 73,950 and 79,070 mg/L, respectively. After screening with a 35-mesh sieve, the TSS concentration was reduced by 30.4% for the swine wastewater and 43.1% for the dairy wastewater. Flocculation experiments were conducted with the prescreened wastewater. only the results using the DS=0.86 sample were presented here. Cationic starch samples with lower DS were found to deliver lower flocculation performance.

The experimental results are listed in Table 2.3. The TSS reduction efficiency was generally greater for dairy (36-58%) than swine wastewater (22-28%). This is likely related to a difference in wastewater compositions. The swine wastewater in this study had a higher pH value (pH=8.6) and total nitrogen content (TN) =6172 mg/L) than the dairy wastewater (pH=6.5 and TN= 1631 mg/L). Such a difference may be caused by differences in feed diet, animals' digestive systems, and waste management systems. For example, due to the use of sand beddings, the wastewater from sand-bedded free-stall dairies contained more minerals (e.g., silicon and carbonates) than swine wastewater (Rabinovich, Rouff, Lew, & Ramlogan, 2018).

Table 2.3. TSS removal from dairy and swine wastewater using the prepared cationic starch.

Dosage (mg/L)	Average TSS (mg/L)	Reduction efficiency (%)	Unit TSS removal [a] (w/w)
Swine wastewater, with initial [TSS] = 51,433 ± 1607 mg/L			
80	40,210 ± 1392	21.8	140.3
125	39,617 ± 1077	23.0	94.5
160	37,517 ± 993	27.1	87.0
200	36,950 ± 1905	27.9	72.4
250	38,417 ± 1487	25.3	52.1
Dairy wastewater, with initial [TSS] = 44,968 ± 104 mg/L			
80	28,750 ± 132	36.1	202.7
125	25,400 ± 379	43.5	156.5
160	18,900 ± 275	58.0	162.9
200	25,850 ± 180	42.2	95.6
250	27,500 ± 29	38.8	69.9

[a] Unit TSS removal is defined as the TSS removal (mg/L) per mg/L of flocculant added in the wastewater.

The dose level of cationic starch had a significant impact on TSS reduction efficiency. For both swine and dairy wastewater, an optimal dose level existed within the studied concentration range (60-250 mg/L); and it was 200 mg/L for swine and 160 mg/L for dairy wastewater. The dose level is a major factor affecting the performance of a flocculant. Both under and over-dosing could result in poor solid-liquid separation (A. Mohseni et al., 2021). Flocculation/coagulation is a complex process and it involves multiple mechanisms such as charge neutralization, sweep coagulation, bridging, and patch flocculation (Suopajärvi, 2015). For cationic starch, the ideal dose level for flocculation is related to the charge density to destabilize the particles and molecular weight of starch polymers to promote sweep flock and inter-particulate bridging. Excessive cationic starch could lead to net positive charges on the solid particle surface, causing electrostatic repulsion and thereby re-stabilizing the suspended solids (Maraschin, Ferrari, da Silva, & Carissimi, 2020). In addition to TSS reduction efficiency, unit TSS removal, which was defined as the TSS removal (mg/L) per mg/L of flocculants added, was also calculated. It decreased with the dose level for both swine and dairy wastewater.

A comparison of the prepared cationic starch with other flocculants for livestock wastewater treatment is given in Table 2.4. The cationic starch did not outperform other flocculants in terms of TSS reduction efficiency. Commercial flocculants, such as polyacrylamide (PAM) and chitosan, can remove >95% of TSS in livestock wastewater upon optimization. The low TSS reduction efficiency for cationic starch is possibly related to prescreening in this study which could have removed many settleable solids. However, in terms of unit TSS removal, the prepared cationic starch outperformed most flocculants. A large unit TSS removal number is critical for the application of a flocculant because most livestock wastewater contains exceptionally high TSS contents and high dose levels are undesired due to cost and water quality considerations. It remains uncertain why the cationic starch delivered superior unit TSS removal, and a further investigation of its flocculation mechanisms is needed.

Table 2.4. TSS removal from livestock wastewater via flocculation: A comparison of several previous studies.

Wastewater	Flocculant [a]	Dosage (mg/L)	Reduction efficiency (%)	Unit TSS removal (w/w)	References
Swine	PAM	350	95.6 [b]	0.48	Walker & Kelley (2003)
	PAM	80-200	37-64 [c]	24-34	González-Fernández, Nieto-Diez, León-Cofreces, & García-Encina (2008)
	SAP	1250	33	0.60	Pamela Chelme-Ayala, Mohamed Gamal El-Din, Richard Smith, Kenneth R. Code, & Jerry Leonard, (2011)
	Cationic starch	200	28	72	This study
Dairy	Chitosan	540	98.7 [d]	5.8	Garcia et al. (2009)
	Cationic hemicelluloses	400	80 [e]	0.67	Pacheco et al. (2022)
	Cationic starch	160	58	163	This study

[a] PAM – polyacrylamide; SAP – superabsorbent polymer. [b] The study tested multiple different swine wastewater. To be relevant, only the results from grower-finisher swine wastewater were included. The TSS concentration of raw wastewater was low (177.3 mg/L). [c] The study tested multiple different swine wastewater. To be relevant, only the results from pre-sieved feeder-to-finish swine wastewater were included. [d] The reduction efficiency was achieved through flocculant addition followed by sieving (with 1-mm screens). [e] The reduction efficiency was achieved at pH = 9.0 The raw wastewater was diluted with a TSS concentration of 336 mg/L.

2.3.4. Circular economy and others

The U.S. livestock industry is proactively pursuing circular economy opportunities to promote sustainability and environmental stewardship. Solid separation is considered a key technology to mitigate odors and recover phosphorus from livestock wastewater (Burton, 2007) To enhance solid separation, commercial metal salt coagulants and organic polymer flocculants such as alum, ferric sulfate, and polyacrylamide (PAM) have been increasingly used. However, these agents are made from minerals (e.g., ferric sulfate) or petroleum (e.g., PAM), or chemicals known to be toxic to ecosystems (e.g., cationic PAM and alum) (Lapointe & Barbeau, 2017; Renault, Sancey, Badot, & Crini, 2009) and, thus, carry relatively large environmental footprints. If one can make a natural flocculant from locally available agricultural waste and recycle it for livestock wastewater treatment, it will significantly reduce the environmental footprint of integrated crop-livestock farming systems. This study represents one of our attempts at facilitating the circular economy for livestock agriculture in the Upper Midwest.

Cationic starch has been explored as a natural flocculant for years. Unlike organic polymer flocculants, it is non-toxic and causes no secondary pollution (Chang, Hu, Wang, & Zhou, 2021). However, only a few studies reported its preparation from potato peels (Lappalainen et al., 2017; Liu et al., 2022) and no application of cationic starch for livestock wastewater treatment was reported. With a modified preparation method, the cationic starch acquired from this study delivered moderate solid reduction efficiency (22-58%), similar to that (21-38% for cationic starch and alike) reported in the literature (Bratskaya, Schwarz, Liebert, & Heinze, 2005; Krentz et al., 2006; Shirzad-Semsar, Scholz, & Kulicke, 2007; Zou, Zhu, Wang, Sui, & Fan, 2011). However, due to different wastewater tests, cations must be taken when comparing the results between studies. Chitosan is extracted from crustacean shells as a fishery and aquaculture byproduct, and it has been extensively studied. However, due to logistic constraints, its application for livestock wastewater treatment in the U.S. Midwest appears to be less promising than cationic starch and alike made from potato peels and other locally available agricultural byproducts.

2.4. Conclusion

Converting agricultural byproducts into value-added products is beneficial for agricultural sustainability. It would be even greater if the products could find agricultural applications, thereby facilitating a circular economy. In this study, we extracted starch from PPW, an abundant agricultural byproduct, and converted it to cationic starch flocculants through a simple wet chemistry process. The preparation of cationic starch samples showed decent performance in the flocculation removal of TSS from swine and dairy wastewater. The TSS efficiency could be improved by further increasing the DS of cationic starch via the use of different catalysts or solvents. Compared to many commercial flocculants, the prepared cationic starch required a relatively low application dosage to remove the same amount of TSS. Since microbes in livestock wastewater are generally negatively charged, efforts will be made to further study the flocculation removal of waterborne Coliforms and antibiotic-resistant bacteria. This study is anticipated to stimulate the valorization of agricultural waste and facilitate the development of eco-friendly, innovative solutions for livestock wastewater management.

CHAPTER 3

Preparation of cationic cellulose as a natural flocculant/sorbent and its application in three water treatment scenarios

The research presented in Chapter 3 has been published in *Water* (MDPI).

DOI: https://doi.org/10.3390/w15112021

Abstract:

In this study, cationic cellulose (CC) was prepared by etherifying commercial cellulose with (3-chloro-2 hydroxypropyl) trimethylammonium chloride (CHPTAC) in an alkaline medium. The prepared CC was characterized using Fourier transform infrared spectrometry (FTIR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and nuclear magnetic resonance (NMR). The characterization results affirmed the successful cationization of cellulose. Upon optimization of reaction conditions, a CC sample with a degree of substitution (DS) of 0.865 was achieved. The CC sample was then tested as a flocculant or sorbent in three environmental applications: algal harvesting, solid removal from dairy wastewater, and capture of methyl orange (MO) in dye wastewater. The effects of dose level and pH on flocculation/sorption performance were studied. Under the optimal dose level and pH conditions, up to 90.4% of dry algal biomass and 53.3% of suspended solids in the dairy wastewater were removed, as measured by standard jar testing. Around 64.2% of MO in the synthetic wastewater was sorbed on the prepared CC and removed, as determined by absorbance at 463 nm. The new CC preparation method exempts the pre-dissolution of cellulose in a solvent and is expected to promote the application of CC in water treatment and similar scenarios.

Keywords: cationic cellulose; flocculant; wastewater; methyl orange; removal efficiency

3.1. Introduction

Cellulose, the most abundant renewable biopolymer, has long been investigated as a green material to substitute non-renewable materials and chemicals. However, its high crystallinity and highly ordered hydrogen bond network detract from the reactivity and solubility of cellulose, hindering its practical applications (Noor Haleem, Arshad, Shahid, & Tahir, 2014; J. Sirviö, Honka, Liimatainen, Niinimäki, & Hormi, 2011). To overcome the constraints, various modification methods (including chemical and physical ones) have been reported. The most prevalent, conventional method is chemical surface treatment through reactions such as carboxylation, esterification, and nitration (Han, Zhu, & Cheng, 2022). Chemical reagents such as alkalis and enzymes have been used to hydrolyze cellulose's crystalline and amorphous region, causing a change in its structure and properties (Du et al., 2016). Efforts have been made, to dissolve cellulose in organic solvents (Lindman, Karlström, & Stigsson, 2010), alkali salts with urea and thiourea (Luo & Zhang, 2013), deep eutectic solvents (Y.-L. Chen, Zhang, You, & Xu, 2019), ionic liquids, and ionic liquids with co-solvents like DMSO and DMAc (Verma et al., 2019a). Then, other reagents are added to react with the dissolved cellulose to produce desired modified cellulose products, e.g., cationic cellulose and nanocellulose membranes (Sjahro et al., 2021; Verma et al., 2019b).

However, the existing procedure has several limitations. The most renowned ones are the non-biodegradability, volatility, toxicity, and high costs of reagents/solvents required (Verma et al., 2019b). Other limitations have also been identified. For example, in NaOH/urea aqueous solutions, the solubility of cellulose is contingent upon the temperature of the solvent (-10 to -12°C), and spinning the solutions containing high concentrations of cellulose is unstable, which poses a challenge for industrial applications (Jin, Zha, & Gu, 2007). Various cationic groups have been successfully added to cellulose backbones. However, no successful use of N-(3-chloro-2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC) to substitute the hydroxyl groups of cellulose has been reported, without first dissolving cellulose in a homogeneous or heterogeneous solvent system. In this study, we developed a new preparation method capable of producing cationic cellulose (CC) with a high degree of substitution (DS) in a cost-effective, environment-friendly manner (Fig. 3.1). We further demonstrated the CC's three environmental applications: algal harvesting, flocculation removal of solids from dairy wastewater, and removal of methyl orange (MO) from synthetic textile wastewater. CC is a highly efficient flocculant that can function effectively across a broad pH range. Furthermore, it is non-toxic and has the added benefit of being readily biodegradable (Hairong Zhang et al., 2016). It has been used for pollutant removal from various types of wastewater (Kono, 2017; Sievänen et al., 2015; Yan, Tao, & Bangal, 2009). This study further compared its flocculation characteristics in different application scenarios.

The dairy industry continues to grow in the Upper Midwestern U.S., raising a substantial challenge to manure management (Glibert, 2020). Dairy wastewater is characterized by high concentrations of total suspended solids (TSS), organic matter, nutrients, and potentially pathogenic microorganisms (Pamela Chelme-Ayala, Mohamed Gamal El-Din, Richard Smith, Kenneth R Code, & Jerry Leonard, 2011). Efficient solid-liquid separation capable of removing a substantial amount of organic solids from liquid or slurry manure can offer multiple benefits. These include the production of nutrient-rich organic solids, odor reduction in subsequent manure storage or treatment units, and improved economics of subsequent treatment processes due to reduced organic loading rates (R. Zhang & Westerman, 1997).

Excess nutrients are associated with the eutrophication of water bodies, causing hazardous algal blooms in aquatic ecosystems. Nutrient recovery through solid-liquid separation, especially with the aid of natural flocculants, can not only avoid depletion of natural resources (e.g., phosphate ores) and water pollution but also promote a circular economy for agricultural production (López-Sánchez et al., 2022). To our knowledge, CC has not been tested for solid-liquid separation from dairy wastewater.

Microalgae have demonstrated great potential in nutrient removal and wastewater treatment (Osabutey et al., 2023). They can be used as animal feed additives, biofuel production feedstock, and dietary or nutraceutical components in human nutrition (Choudhary et al., 2020; Nanda et al., 2021). A challenge to the microalgal bioeconomy is the lack of cost-efficient harvesting methods. Harvesting was estimated to account for 20–30% of the total algal biomass production cost. Technologies including flotation, sedimentation, filtration, flocculation, and centrifugation have been attempted (Jaiswal et al., 2022; Lozano-Garcia et al., 2019; Mennaa, Arbib, & Perales, 2015). Despite its great performance, the application of flocculation is impeded by difficulty in separating flocculants from harvested microalgal biomass. Specifically, common flocculants like aluminum sulfate, ferric sulfate, and polyacrylamide (PAM) are hard to remove from the settled flocs, and the presence of these flocculants poses a challenge to the downstream processing or utilization of algal biomass. Natural or bio-flocculants are therefore being actively pursued as substitute reagents (Okoro, Azimov, Munoz, Hernandez, & Phan, 2019).

The wastewater from the textile manufacturing industry can contain high concentrations of unused dyes, as well as surfactants and other organic compounds (X. Yang & Ni, 2012). Various treatment methods, such as coagulation/flocculation, photocatalytic, advanced oxidation, and biological wastewater treatment (including both aerobic and anaerobic) have been attempted for dye removal (Jjagwe, Olupot, Menya, & Kalibbala, 2021; Lau et al., 2015). Among them, coagulation/flocculation is considered one of the most practical methods (Teh et al., 2016). To eliminate secondary pollution induced by chemical flocculants, CC as a natural flocculant was used to remove MO, a prevalent synthetic dye, from wastewater through its complexation with MO's sulfonate group – a process labeled as flocculation or sorption in the literature. Only one study reported MO removal with modified CC, but the maximum sorption capacity was only 16.94 mg/g (Lafi, Abdellaoui, Montasser, & Hafiane, 2022).

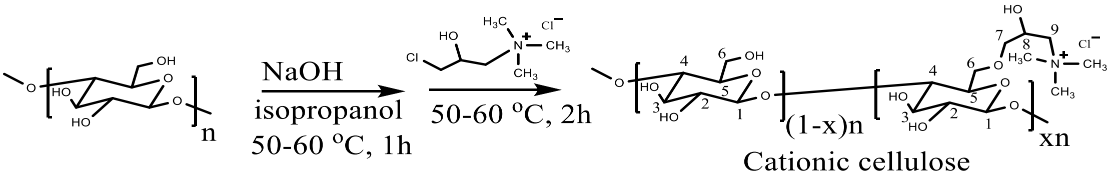


Figure 3.1. Preparation of cationic cellulose. In the first step, any of the OH groups may be deprotonated by NaOH. In the product's structure, the ammonium unit may be attached to the oxygen on C2, C3, or C6.

3.2. Materials and methods

3.2.1. Materials

Microcrystalline cellulose (d = 1.5 g/cm^3) was purchased from MarkNature (Fullerton, California). Sigma-Aldrich (St. Louis, Missouri) supplied the cationization reagent, CHPTAC (60 weight percent in water, with d = 1.154 g/mL, molecular weight [MW] = 188.10 g/mol), isopropanol (91%), ethanol (99.5%), and D2O. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Fisher Scientific (Hampton, New Hampshire). All the reagents except for the cellulose were of analytical grade and were used as received without further purification.

3.2.2. Preparation of cationic cellulose

Five grams of commercial cellulose were placed into a 400 mL beaker and mixed with 200 mL of isopropanol. The mixture was stirred at 300 rpm for 30 minutes at room temperature. After that, 5.0 mL of a 1-4 M, NaOH solution was added dropwise into the reaction mixture at 50-60^{o}C. The mixture was then stirred for another hour at the same temperature in the alkaline medium. For etherification, 3-10 mL (0.053-0.18 M; depending on the experimental setting) of CHPTAC (60 wt%) was added to the alkalized cellulose at 60°C under vigorous stirring and the heat stirring continued for two more hours. After the mixture solution cooled down to room temperature, isopropanol evaporated. The remaining part (cellulose plus water) was then stirred and filtered using a glass fiber membrane filter paper (pore size = 1.6 µm). The solid collected was washed several times using ethanol (99.9%) to remove unreacted reagents and byproducts. The final product was air-dried overnight at 40°C in a lab oven.

3.2.3. Characterization of cationic cellulose

A thin coating of gold was applied onto a double-sided adhesive carbon tape containing a sample weighing approximately 1.0 mg. The tape was then examined using an accelerating voltage of 10.0 kV, and images were captured at different levels of magnification to investigate the size and shape of the cellulose granules. Fourier-transformed infrared spectroscopy (FT-IR) analysis was performed using a Perkin-Elmer Spectrum 100 FT-IR spectrometer. CC powder and KBr were combined in a 2:98 (w/w) ratio, pressed into a transparent disk, and scanned 28 times at a resolution of 4 cm^{-1} to obtain a transmittance spectrum. The primary objective of this analysis was to identify the functional groups that indicate the successful modification of cellulose (Zaman, Xiao, Chibante, & Ni, 2012).

Differential scanning calorimetry (DSC) was conducted using a Perkin-Elmer DSC 6000 analyzer. Approximately 10 mg of the CC sample was loaded into an aluminum sample pan, purged with N2 at a flow rate of 20 mL/min, and subjected to heating from 20 to 450°C at a ramp rate of 10°C/min. An empty pan used as a reference allowed for signal subtraction. The analysis aimed to compare the original cellulose with CC in terms of their glass transition temperature (Tg) and decomposition temperature. Any shift in these temperatures would indicate a successful modification. Nuclear magnetic resonance (NMR) specifically proton NMR (1H NMR), was used, to identify functional groups indicative of successful cationization. The analysis was conducted using a Bruker Avance 300 MHz NMR spectrometer. CC samples were dissolved in D2O, which was used as an internal standard. Chemical shifts in parts per million (ppm) were obtained using MestReC software. These analytical techniques were employed to characterize the morphology, functional groups, thermal properties, and chemical structure of the cationic cellulose sample, providing insights into the success of the modification process.

3.2.4. Degree of substitution (DS)

DS is defined as the average number of hydroxyl groups substituted by quaternary ammonium groups at C-2, 3, and 6 in the backbone of cellulose. The nitrogen content of the cationically modified cellulose was determined using the TNT plus 826 test kit (Hach Method 10208) (L. Li, 2012). DS was calculated from the nitrogen content in cationically modified cellulose with Eq. 3.1.

$$DS = \frac{162 \times \mathrm{N}}{1400 - CR \times N} \tag{3.1}$$

Where, 162 = molecular weight of anhydrous glucose unit (AGU), N = percentage of nitrogen as determined by the nitrogen analysis, and CR = molecular weight of the cationic reagent.

3.2.5. Harvesting of microalgae by flocculation

Scenedesmus dimorphus UTEX 1237 was cultivated in the Bold’s Basal Medium (BBM), as described by (Osabutey et al., 2023). Dry algal biomass (DAB) concentration (mg/L) was determined gravimetrically by vacuum-filtering 5 mL of microalgal suspensions through a 90-mm glass fiber filter, drying the microalgae-laden filter at 105°C for one hour, weighing the filter on a microbalance, and subtracting the reading from the weight of the empty filter (Deepa, Keerthana, Pratheep Kumar, & Suryaprakash, 2022; Vandamme, Foubert, & Muylaert, 2013). The initial DAB concentration was 1950 mg/L before the flocculation experiment. The flocculation experiments were conducted using a six-paddle jar tester (Phipps & Bird Model 7790-901B; Fisher Scientific Inc., Richmond, Virginia) following, a standard protocol described by Ref. (Marinho et al., 2022). First 1.0 g of CC was mixed with 100 mL of deionized water and stirred for 30-40 minutes. The resulting flocculant solution was then added to 0.5 L of pre-screened algae culture broth to achieve the desired dose levels (80, 125, 150, 200, and 250 mg/L). The experiment was replicated three times for each dose level using jar tests. Before adding the flocculants, the pH of the microalgal culture broth in each jar testing beaker was 6.5. Coagulation was stimulated by intense stirring at 300 rpm for one minute, followed by gentle stirring at 20 rpm for 20 minutes to promote flock formation. The mixtures were given a 24-hour settling period to allow the sedimentation of the flocs. Subsequently, liquid samples of approximately 5 mL were extracted from each beaker, with the sampling point approximately 2 cm below the surface. The DAB concentrations in the samples were determined gravimetrically as previously described.

3.2.6. Flocculation removal of TSS from dairy wastewater

The flocculation experiment was conducted on a six-paddle jar tester following the procedure discussed in Section 2.5. Samples of wastewater were collected from a free-stall dairy near Lake Benton, Minnesota with around 2000 lactating cows. Sand lanes were used at the facility to separate sand bedding from manure wastewater. The samples were taken after the separation process. It is noteworthy that one of our previous studies [38] collected wastewater samples from the same facility but a year before the current study. Waste quality parameters including TSS were therefore slightly different.

3.2.7. Complexation removal of methyl orange (MO)

One gram of MO was dissolved in 1 L of deionized water and was further diluted to 100 ppm (1 ppm = 1 mg/L). To build a calibration curve, serial dilution to 2, 4, 6, and 8 ppm was performed. A 50 ppm MO solution was prepared from the 100 ppm stock solution to assess the complexation removal of MO by CC. Several factors were investigated, including the dose level of CC (50, 100, 150, 200, 250, and 300 ppm), pH (4-10), reaction temperature (20-60°C), and reaction time (1-3 hours). The solution was constantly stirred during the test. The pH of the solution was adjusted with 0.5M NaOH and HCl and was recorded using a pH meter. Upon the completion of the reaction, the solution was centrifuged at 6000 RPM for 10 minutes. The supernatant was diluted by deionized water at a dilution ratio of 10 and measured for MO concentrations at 463 nm using a visible light spectrophotometer (Spectrophotometer 721; Huanghua Faithful Instrument Co., Ltd, Changzhou, China) at 463 nm. MO removal efficiency (%) was calculated with Eq. 3.2.

$$\text{MO reduction (\%)} = \frac{\mathrm{C_0} - \mathrm{C_t}}{\mathrm{C_0}} \times 100 \tag{3.2}$$

where C_0 and C_t are the initial and final MO concentrations, respectively. Dye sorption capacity (q_e) was calculated as:

$$q_e = \frac{(C_0 - C_t) \times V}{m_{cc}} \tag{3.3}$$

Where V is the volume of the MO solution, and m_{cc} is the weight of CC added to the solution.

3.3. Results and discussion

3.3.1. Structural analysis

A total of thirteen CC samples were prepared with different DS values. They were acquired by adjusting the preparation conditions such as NaOH concentration, CHPTAC concentrations, and reaction time. It was observed that the concentration of NaOH was the most significant factor. factor was found to be the NaOH concentration. DS reached its maximum value (0.865) as the NaOH concentration was increased to 3.0 M (with 0.124 M CHPTAC). Further increasing the NaOH concentration led to a slight decrease in DS values. The nucleophilicity of the hydroxyl groups in the cellulose backbone increases during the etherification of cellulose using cationic reagents and a strong base like NaOH. The extra hydroxyl ions that NaOH releases may push the reaction equilibrium toward the product side. However, high NaOH concentrations may cause side reactions between the cationic reagent and sodium hydroxide, which can slow down the cationization processes. For simplicity, only two samples (DS=0.525 and DS=0.865) were submitted for structural analysis to verify the successful cationization of cellulose.

FT-IR spectrum

The incorporation of the quaternary ammonium group into the cellulose backbone was confirmed by FT-IR (Fig. 3.2). In both the modified and unmodified cellulose samples, several characteristic peaks were detected. These included a prominent peak at 3457 cm^{-1} representing the stretching vibration of the hydroxyl (-OH) (Sang & Xiao, 2009); a peak at 2901 cm^{-1} associated, with the symmetrical vibration of the C-H bond (Jahan, Saeed, He, & Ni, 2011); and a peak at 1645 cm^{-1} primarily attributed to moisture absorbed by the cellulose samples (Zaman et al., 2012). Different from the unmodified sample, the modified samples showed a peak at 1484 cm^{-1}. The vibration of the C-N bond in the quaternary ammonium groups can be attributed to this, indicating the successful integration of trimethyl ammonium groups (Noor Haleem, Osabutey, et al., 2023). Furthermore, the absorption peak observed at 839 cm^{-1} was attributed to the deformation of the C-H bond within the glycosidic bond that connects the glucose units (Loubaki, Ourevitch, & Sicsic, 1991; Yan et al., 2009).

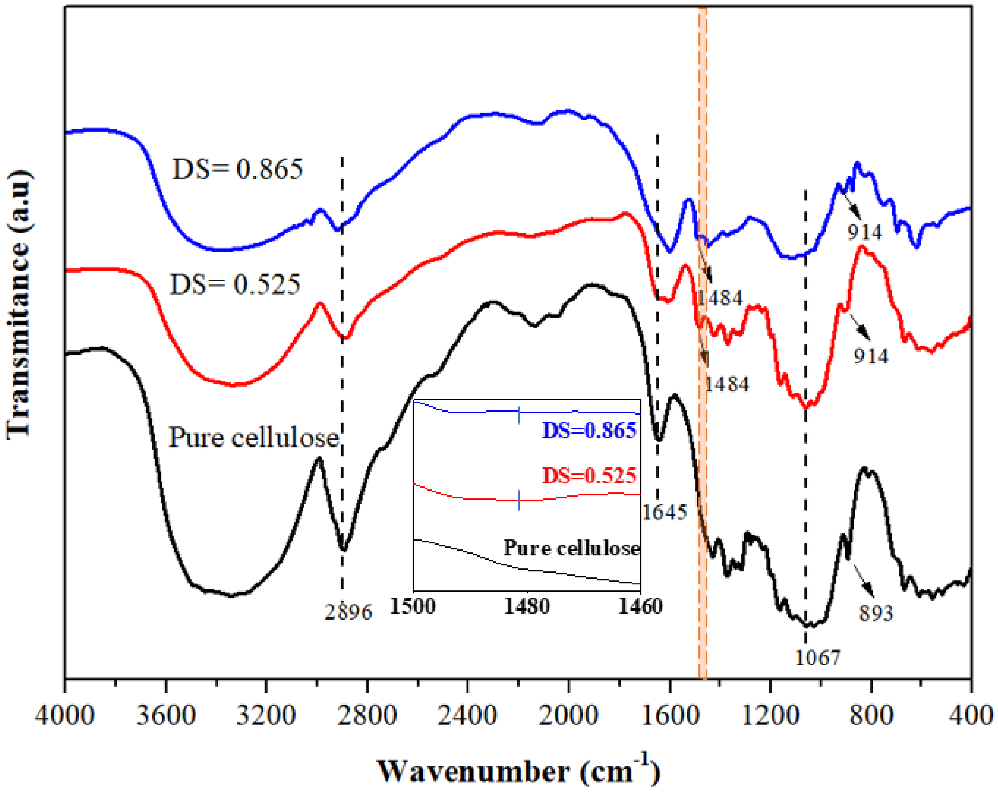


Figure 3.2. FT-IR spectra of pure cellulose and cationic cellulose samples. Note: The relatively weak 1484 cm-1 peak of the DS=0.865 sample was due to the deamination of CC after long-term storage.

DSC thermogram

DSC analysis results supported the incorporation of cationic groups into the backbone of the cellulose (Fig. 3.3). The endothermic and exothermic peaks were ascribed to the evaporation of levoglucosan formed from cellulose depolymerization, while the exothermic peaks were attributed to char formation (J. A. Sirviö et al., 2014). The glass transition (T_g) temperatures of the DS=0.525 sample (78.2°C) and the DS=0.865 sample (81.0°C) were both slightly lower than that of unmodified cellulose (84.6°C). DS exhibited a large influence on the thermal stability of the prepared CC samples. The DS=0.865 sample decomposed at a slightly lower temperature (210-248°C) than the DS= 0.525 sample (231-252°C). Comparatively, the decomposition temperature of unmodified cellulose was 351°C, indicating degraded thermostability after the cationic alteration of cellulose. The changes in DSC peaks after modification might have also resulted from changes in cellulose morphology and crystallinity levels before and after cationization reactions. Similar findings were reported in the literature (Chirila, Popescu, Cutrubinis, Stanculescu, & Moise, 2018; G. Li, Fu, Shao, Zhang, & Qin, 2015; F. Zhang, Pang, Dong, & Liu, 2015).

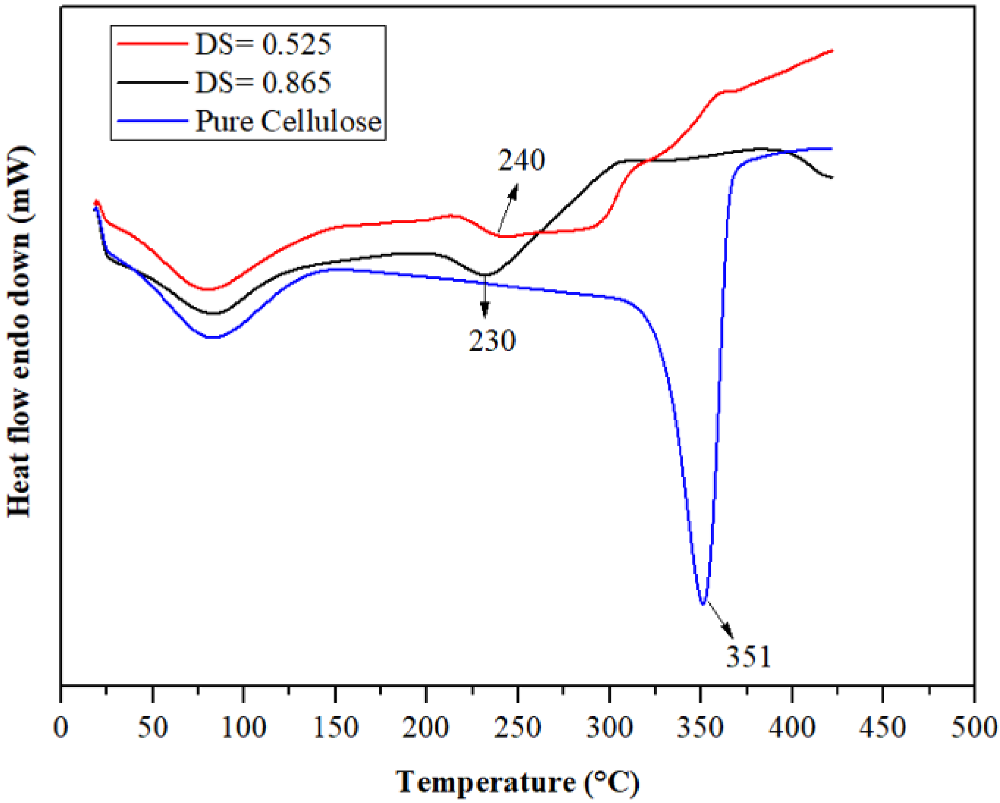


Figure 3.3. DSC thermograms of pure cellulose and cationic cellulose samples.

NMR spectrum

Certain challenges were encountered during NMR sample preparation. The samples were prepared by stir-mixing 0.2 g CC with 20 mL of deionized water. However, it resulted in a stable colloidal solution, and no solid settling was seen even after two days. To address the issue, the colloidal solution was further filtered with a 0.2 µm (in pore size) filter to a pre-weighed NMR tube. However, the filtrate remained cloudy. The water in the NMR tube was removed via vacuum drying (20 mbar and ~60°C) until the mass of the solid residue became constant. D_2O was then added to the NMR tube and the tube was heated to ~90°C for 30 seconds. Both H NMR and C NMR analyses were performed, but no signal was obtained during the C NMR analysis. The ^1H NMR spectra of the D_2O soluble portion of the two CC samples are shown in Fig. 3.4. The hydrogen deuterium oxide (HDO) signal at 4.80 ppm was used as the reference for chemical shifts, while the H_2O peak occurred at 4.81 ppm. The peak of the three methyl groups of the ammonium appeared at 3.27 ppm, and the integration of the peak was set to be 9H. The signals between 3.31 and 4.71 ppm were attributed to the Hs on the carbons of cellulose and carbon linkages between the ammonium group and the cellulose backbone. The integration of this shift range was 18.32H. The signal around 6.5 ppm and between 1.6 and 2.3 ppm became stronger with an increase in DS.

A stronger reaction condition or a longer reaction time would lead to more deamination-producing methyl ketone moieties. The moieties would further react among themselves to produce groups like $C=C-CH_3$ and $CH_3-CHR-OH$ that were seen in the regions mentioned above. The reactions could occur among modified cellulose polymers, leading to the formation of sub-micron cross-linked particles. These submicron colloidal particles did not aggregate due to the presence of positive ammonium groups on their surface. This explains the cloudiness of the acquired CC solutions and their persistency – the cloudiness did not disappear upon dilution or heating. The DS of the prepared CC sample was also calculated from the NMR results using Eq. 3.4:

$$\mathrm{DS} = \frac{9/9}{(I_{H\,on\,C1-9} - 5)/7} \tag{3.4}$$

Where the first 9 is the integration set for the methyl peak, the second 9 is the number of hydrogen atoms in one ammonium unit, $I_{H\ on\ C1\text{-}9}$ is the integration of signals of H atoms on carbons 1-9, 5 is the number of H on C (7-9), and 7 is the number of H on C (1-6) of an AGU. In this calculation, OH signals were not considered because they do not show up in the spectra due to deuteration by D_2O. The calculated DS values are 0.525 and 0.865, respectively, the value is close to that measured through nitrogen analysis.

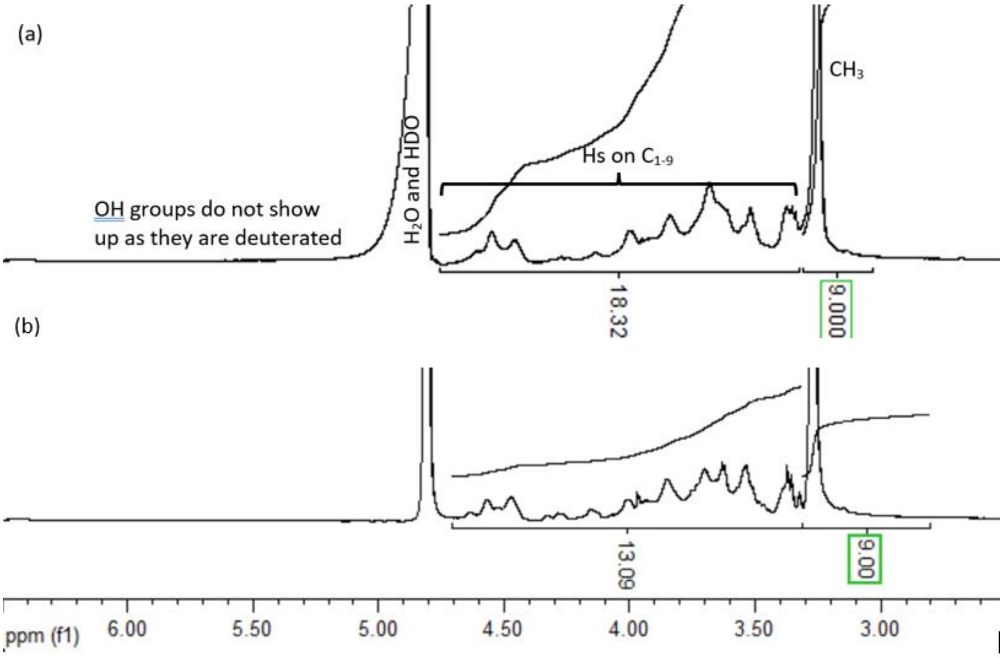
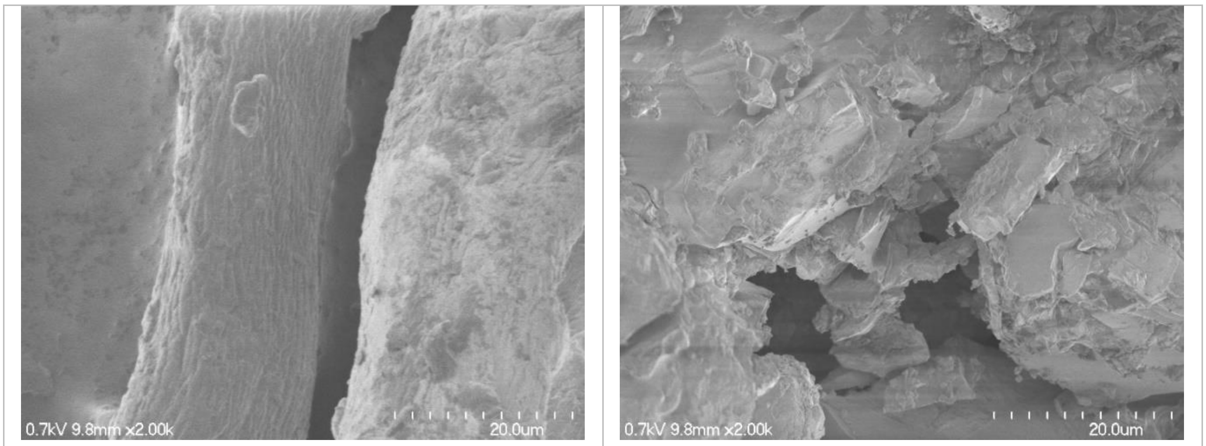


Figure 3.4. 1H NMR spectra of two cationic cellulose samples: (a) DS=0.525 and (b) DS=0.865. Note that the water peak in (a) is much stronger, which reflects the lower solubility of the DS=0.525 sample in D_2O as a result of the lower DS.

Scanning electron microscopy

The unmodified cellulose showed a regular cellulose structure (Fig. 3.5), with individual fibers with smooth surfaces depicting less accessibility to the cationic reagent. After cationization, the morphology of the CC sample changed drastically, featuring a loose and porous structure. This is ascribed to the incorporation of quaternary ammonium groups (Fig. 4 (b)), suggesting that the surface area of cellulose significantly increased. Materials with loose and porous structures are preferred for adsorbing and flocculating contaminants from water (Obey, Adelaide, & Ramaraj, 2022; Zhen Yang et al., 2013; F. Zhang et al., 2015) . Thus, it is anticipated that the prepared CC samples could deliver superior flocculation/sorption efficiency.



(**a**) (**b**)

Figure 3.5. SEM images of (a) pure cellulose fibers and (b) cationic cellulose with DS=0.865.

3.3.2. Flocculation experiments

Algal harvesting

For algal harvesting, several CC samples differing in DS values were tested but the only results of using the DS=0.865 CC sample were presented here due to its best performance. The initial concentration of DAB was 1,950 mg/L, while the pH of the algal culture measured 6.40. When the CC was added to the beakers containing the algal culture broth, there was a noticeable separation of solid particles settling at the bottom. However, in the control samples where no CC was added, there was no stratification observed. This demonstrates that CC can be a highly effective flocculant for algal harvesting.

To further optimize the flocculation process, several CC dose levels were tested. DAB removal efficiency increased with the tested dose levels but started to taper offer when the dose level exceeded 200 mg/L (Table 3.1). High flocculation efficiency at relatively low dose levels (several hundred mg/L) is characteristic of polyelectrolyte flocculants like PAM and polystyrene sulfonic acid (Pugazhendhi et al., 2019; Van Haver & Nayar, 2017). This study showed that CC behaves similarly to other polyelectrolyte flocculants. The best flocculant dose level depends on the properties of the microalgal suspension as well as flocculation mechanisms (Lopez-Exposito, Campano, van de Ven, Negro, & Blanco, 2019). Influential factors may have a significant impact, such as the pH, chemical composition of the algal culture broth, the size and concentration of microalgae, the charge density on the microalgal cell surface, and the charge density of the flocculant (Philip A Hansel, Riefler, & Stuart, 2014).

Table 3.1. Flocculation of *Scenedesmus dimorphus* with prepared cationic cellulose at different dose levels.

CC dose level (mg/L)	Initial algal biomass (mg/L)	Final algal biomass (mg/L)	Removal of dry algal biomass (%)
80	1950 ± 550	1016 ± 103	47.9
125	1950 ± 550	766 ± 103	60.7
160	1950 ± 550	450 ± 141	76.9
200	1950 ± 550	267 ± 24	86.3
250	1950 ± 550	183 ± 47	90.6
300	1950 ± 550	133 ± 24	93.0

For CC, the associated flocculation mechanisms include interparticle bridging and charge-patch. During interparticle bridging, fragments of dissolved cellulose polymer chains adhere to the surface of microalgal cells, while the rest of the chain extends into the surrounding solution, forming tails or loops. These loops and tails can then bind to other cells (Abbasi Moud, 2022). Mixing promotes collision and accordingly floc formation. In charge-patch, electrostatic interactions drive the positively charged CC polymers to the negatively charged microalgal cell surface. This creates a localized zone of charge inversion that subsequently attracts nearby algal cells with opposite charges (Pugazhendhi et al., 2019). Since pH affects both the surface charge of microalgal cells and the charge of CC polymers, a significant effect of pH was observed (Table 3.2). The finding is consistent with an earlier report (Zijun Yang, Hou, & Miao, 2021).

Table 3.2. Effects of pH on removal efficiency (RE).

pH	Microalgae		Dairy wastewater		Methyl orange	
	Final DAB (mg/L)	RE (%)	Final TSS (mg/L)	RE (%)	Final MO (mg/L)	RE (%)
4	495 ± 212	74.6 ± 5.3	24303 ± 100	53.4 ± 1.9	27.7 ± 1.8	44.5 ± 2.1
5	313 ±110	84.0 ± 5.3	23216 ±126	54.8 ± 1.4	20.7 ± 4.1	58.7 ± 5.0
6	250 ± 126	87.2 ± 7.2	23516 ± 122	54.3 ± 1.2	17.3 ± 0.5	65.3 ± 0.7
7	181 ± 174	90.5 ± 1.2	23545 ± 358	54.2 ± 2.1	17.9 ± 1.3	64.3 ± 1.6
8	178 ± 420	90.9 ± 1.2	24860± 178	51.8 ± 0.3	24.7 ± 2.2	50.7 ± 2.6
9	228 ± 112	86.3 ± 2.4	29100 ± 187	43.5 ± 0.6	28.0 ± 2.7	44.0 ± 1.7
10	816 ± 532	58.2 ± 1.2	32850 ± 161	36.2 ± 0.8	42.0 ± 4.1	16.0 ± 3.0

Solid-liquid separation of dairy wastewater

Flocculation experiments were performed with prescreened (using a 35-mesh sieve) dairy wastewater with a TSS concentration of 51,466 mg/L and a pH value of 6.6. Only the results of the DS=0.865 sample were presented here (Table 3.2 and Table 3.3). The TSS reduction efficiency for dairy wastewater (47-53%) was lower than the DAB reduction efficiency for algal culture (47-90%). The difference in their solid compositions may account for this variation. The optimal dose level within the studied concentration range was 160 mg/L. Both under and over-dosing may lead to ineffective separation of solid-liquid (F. Mohseni & Moosavi Zenooz, 2022).

Table 3.3. Removal of TSS from dairy wastewater with prepared cationic cellulose at different dose levels.

CC dose level (mg/L)	Initial TSS (mg/L)	Final TSS (mg/L)	TSS removal (%)
80	51466 ± 675	26685 ± 900	47.1
125	51466 ± 675	25735 ± 369	49.9
160	51466 ± 675	25350 ± 444	50.5
200	51466 ± 675	24270 ± 101	53.3
250	51466 ± 675	24700 ± 180	52.3

A natural flocculant that outperformed the CC was a commercial chitosan product with a 73% TSS reduction (Geetha Devi, Dumaran, & Feroz, 2012). However, these two flocculants are not directly comparable. While for CC the positive charge is attributed to quaternary ammonium groups, for commercial chitosan flocculants like chitosan acetate, it is caused by the protonation of amino groups (Rolf et al., 2021). Chitosan is extracted from crustacean shells as a fishery and aquaculture byproduct, and it has been extensively studied. However, due to logistic constraints, its application for livestock wastewater treatment in the U.S. Midwest appears to be less promising than CC and alike made from locally available agricultural byproducts.

The process of coagulation/flocculation is intricate, involving various mechanisms like charge neutralization, sweep coagulation, inter-particulate bridging, and patch flocculation (Pan, Wang, Mei, & Tang, 2022). For CC, the ideal dose level for flocculation is related to charge density to destabilize the particles and the molecular weight of CC polymers to promote sweep coagulation and inter-particulate bridging. Excess CC could lead to net positive charges on the solid particle surface, causing electrostatic repulsion and thereby re-stabilizing the suspended solids (Noor Haleem, Osabutey, et al., 2023). The effect of pH was also substantial (Table 2). The greatest TSS reduction occurred at the pH range of 5-6. The molecular structure of cellulose could be damaged in a highly acidic environment, which may explain the decreased TSS removal below pH=5 (Zhai et al., 2022). On the other hand, exposure to a high pH environment for an extended time may result in the alkaline hydrolysis of cellulose. The pH of raw dairy water is 8.0. Changes in pH may also alter the constituents of dairy wastewater, thereby affecting the flocculation efficiency. A similar observation was reported by Muniz et al. (Muniz, Borges, & Silva, 2020).

Methyl orange

Effective removal of MO from the synthetic wastewater was achieved with the prepared CC (DS=0.865) (Table 4). The dose level showed a large influence on MO removal. Raising the CC concentration from 50 to 200 mg/L resulted in an increase in MO removal efficiency from 24.3 to 51.8%. It could be ascribed to an increased number of sorption sites at elevated CC concentrations. However, a slight decrease in MO removal efficiency was observed when the dose level was over 200 mg/L, possibly due to the aggregation of CC decreasing the number of effective sorption sites.

The impact of pH on the removal of MO was further studied by fixing the dose level at 50 mg/L (Table 3.4). The greatest MO removal occurred at pH=6. Upon dissolved in water, MO dissociates into Na^+ and MO ions. The sulfonate group ($R-SO_3^-$) is the only part of the MO ion structure that is hydrophilic; the rest is hydrophobic. MO and CC interact primarily through the complexation of -SO_3^- in MO with the quaternary ammonium group in CC (H. Chen, Zhong, Wu, Zhao, & Yan, 2012). Hydrogen bonding between -OH in CC and tertiary amine in MO is another contributor to the interaction. A decrease in pH from neutral (pH=7) would result in increased availability of R-SO_3^- groups. However, MO becomes less soluble at pH<3.5. As a result, the MO removal efficiency decreased when the pH dropped below 4 (Duan et al., 2015). The MO-CC complexation weakens in an alkaline environment (pH>7) because MO would primarily exist in the non-protonated form. It still offers weak electrostatic attractions to CC but no more strong hydrogen bonding remains. Furthermore, with an increased pH, OH^- starts to compete against MO for positively charged active sites in CC, which also causes a decline in MO removal (Lafi & Hafiane, 2016).

Table 3.4. Methyl orange removal from synthetic wastewater with prepared cationic cellulose at different dose levels.

CC dosage level (mg/L)	Initial MO (mg/L)	Final MO (mg/L)	MO removal (%)	q_e (mg/g)
50	50	35.7 ± 2.2	24.3	243
100	50	31.0 ± 5.0	27.9	140
150	50	22.8 ± 0.7	53.0	177
200	50	22.5 ± 1.6	51.8	130
250	50	22.6 ± 2.6	49.4	99

3.4. Conclusions

CC was prepared from commercial cellulose via homogeneous etherification of cellulose with CHPTAC in an alkaline medium. CC samples with different DS values were obtained by adjusting reaction parameters. The optimal reaction conditions (for a maximized DS) were: 4-hour reaction time (alkalization & etherification), 0.124M of CHPTAC, 3 M of NaOH, and 60°C reaction temperature. Structural characterizations (FT-IR, DSC, SEM, and NMR) evidenced the successful introduction of a quaternary ammonium group to the cellulose backbone. A prepared CC sample with DS=0.865 was tested as a flocculant/sorbent in three application scenarios. It delivered decent efficiency in capturing/removing algal biomass (up to 93.0%), TSS (up to 53.3%), and MO (up to 53.0%) from microalgal cultural broth, dairy wastewater, and synthetic dye wastewater, respectively. The efficiency can be further improved by altering the pH and the CC dose level. This study demonstrated the great potential of CC in wastewater treatment applications and is anticipated to promote further exploration of natural flocculants.

CHAPTER 4

DC-initiated flocculation of *Scenedesmus dimorphus*

The research presented in Chapter 4 is under review by *Bioresource Technology*

Abstract:

Despite their superior efficiency, chemical flocculants introduce foreign chemicals, such as metals, into harvested microalgae, posing downstream application challenges. To overcome this, a simple DC-initiated flocculation technology is proposed for microalgal harvesting. This method applies a DC electric field across titanium plate electrodes to promote microalgal cell aggregation by polarizing their electrical double layer. *Scenedesmus dimorphus* cultivated in Bold’s Basal Medium was tested under various voltage gradients (58, 116, 174, and 233 V/m) and treatment durations (20, 40, and 60 min with DC applied), resulting in up to 94% flocculation efficiency based on dry algal biomass, significantly higher than controls. Microbubbles formed due to water electrolysis, but their impact on algal cell separation was minimal. Unlike electrocoagulation, DC-initiated flocculation uses inert electrodes and, thus, introduces no Fe or Al ions. This study is anticipated to facilitate the research on electrochemically assisted algae harvesting and processing technologies.

Keywords: Microalgae; flocculation; harvesting; electric double layer; polarization

4.1. Introduction

Due to their high yield and efficient photosynthesis, microalgae are widely regarded as a promising feedstock material to produce biofuels and value-added products such as pharmaceuticals and cosmetics. However, challenges persist in harvesting microalgal biomass from suspended culture systems. These difficulties arise from factors, such as the small size of algal cells, their strong affinity for water, low density, and negative cell surface charge (Noor Haleem, Zhang, et al., 2023). The expensive process of harvesting microalgal biomass poses a major obstacle, accounting for a significant portion of overall production costs (G. Muhammad et al., 2021). Another challenge lies in foreign materials introduced during microalgal harvesting, e.g., chemical flocculants for enhanced gravitational or mechanical separation. These challenges impede the feasibility of large-scale production as a renewable energy alternative (Fayad et al., 2017). To mitigate these challenges, there is a need for a cost-effective and contamination-free harvesting method.

Various methods are employed for harvesting microalgae, including physical techniques such as filtration, centrifugation, and flotation (Tan et al., 2020). Chemical methods involve the use of coagulants, flocculants, and adsorbents like magnetic nanoparticles, as well as pH adjustment as the pH of the growth medium significantly impacts the performance of these chemicals (Ummalyma et al., 2023). However, the use of chemicals raises concerns about impurities such as heavy metals in obtained algal biomass. The presence of polymeric flocculants also has the potential to alter the carbon profile of microalgae, limiting their range of applications (Fasaei, Bitter, Slegers, & van Boxtel, 2018). Electrocoagulation (also referred to as electro-flocculation) is another chemical harvesting method of interest (Shuangxi Li et al., 2020). Specifically, electrocoagulation employs anodes like aluminum (Al), iron (Fe), magnesium (Mg), copper (Cu), and zinc (Zn) that are gradually consumed during the harvesting process. Different electrodes exhibit varying efficiencies. For example, aluminum electrodes generate Al^{3+} and OH^- ions, leading to the formation of 'sweep flocs' with a significant surface area. However, it releases metal ions that precipitate together with microalgal cells, posing challenges for downstream processing, especially in pharmaceutical applications (Krishnamoorthy et al., 2021).

This study presents a simple DC-initiated flocculation technology for microalgal harvesting (Fig.4.1). It involves the application of a DC voltage across two parallel plate inert electrodes to polarize (deform) the electric double layer (EDL) surrounding suspended microalgal cells. The polarization leads to the formation of electric dipoles and therefore induces dipole interactions, including both attraction and repulsion (Shih et al., 2015). The electrostatic attractive forces facilitate the collision and subsequent coalescence of microalgal cells, thereby initiating the flocculation process. A similar approach has been well-established for destabilizing colloidal suspensions (Dobnikar et al., 2013). In addition to induced dipole attractions, the electrohydrodynamic convection of suspended particles in a DC field may also play a role in particle assembly (Dommersnes et al., 2013), potentially enhancing flocculation efficiency. For water treatment, DC-initiated flocculation was first introduced by a group of researchers in Japan (Mori et al., 2019). However, their research focused on clay-laden synthetic wastewater or mineral dressing wastewater. To our knowledge, this technology has not been explored for microalgae suspensions or organic wastewater treatment. Our investigation demonstrated the viability of employing DC-initiated flocculation for the harvesting of *Scenedesmus dimorphus*, a lipid-rich freshwater microalga. This simple method eliminates the use of chemical additives and reactive electrodes, thus offering potentially reduced harvesting costs.

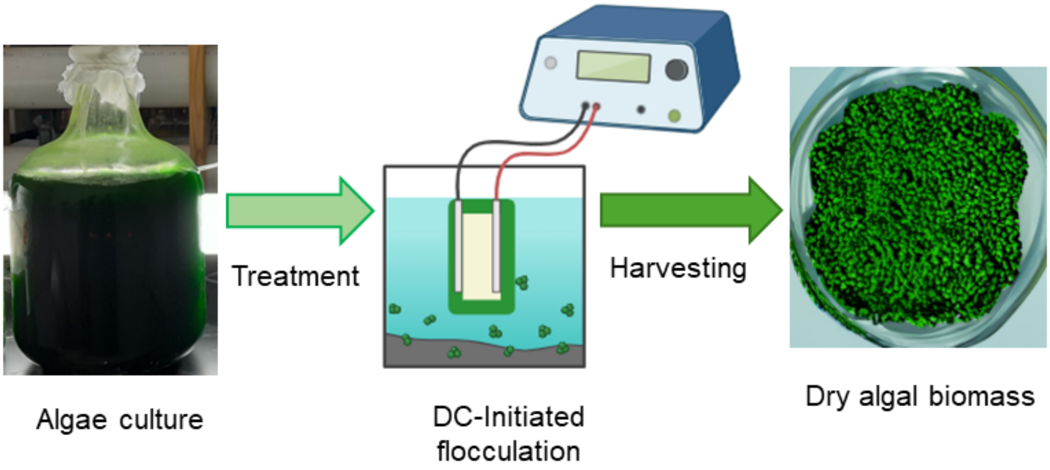


Figure 4.1. DC-initiated flocculation technology for microalgal harvesting.

4.2. Materials and methods

4.2.1. Cultivation of microalgae

The cultivation followed the same protocol as reported by Osabutey et al. (Osabutey et al., 2023). *Scenedesmus dimorphus* strain UTEX 1237 was cultivated using bold basal medium (BBM) at a pH range of 7.0±0.5. Initially, autoclaved BBM was blended with *Scenedesmus dimorphus* seeds. The culture was subsequently transferred to 20 L Pyrex glass bottles, with periodic addition of autoclaved BBM and deionized water. Continuous aeration was maintained at a rate of 0.5 L/min per liter, accompanied by a light intensity of 60–70 $\mu mol/m^2 \cdot s^2$ using white, fluorescent lamps.

4.2.2. Flocculation experiments

The microalgal suspensions obtained from the aforementioned step were set still for at least 24 hours, allowing large microalgal cells or cell agglomerates to settle down. The liquid portion (with a volume of 250 mL) was transferred to a 500 mL beaker equipped with two titanium plates serving as electrodes. Subsequently, a DC voltage of 5, 10, 15, or 20V (equivalent to a voltage gradient of 58.1, 116.3, 174.4, or 232.6 V/m) was applied across the electrodes for varying durations of 20, 40, 60, and

120 min (energizing time). During the duration of treatment, the microalgal suspension was stirred using a 40-mm magnetic stir bar at 250 RPM. After the DC treatment, the suspension remained undisturbed for 60 min, allowing flocs to settle down, before being submitted for microalgal concentration determination. Before each experiment, the electrode surface was rinsed with hydrochloric acid and deionized water to remove surface deposits. The spacing between the two parallel plate electrodes was 8.6 cm. Four beakers were employed in each run of experiments, including a control sample (0 V; without DC applied) and three replicate samples (with DC applied). The flocculation efficiency was averaged from triplicate dry algal biomass (DAB) measurements of each replicate sample. The formation of flocs observed in DC-treated samples, as illustrated in Fig. 4.2 and Fig 4.3. After 60-min gravitational settling, supernatants were sampled approximately 15-20 mm below the liquid surface.

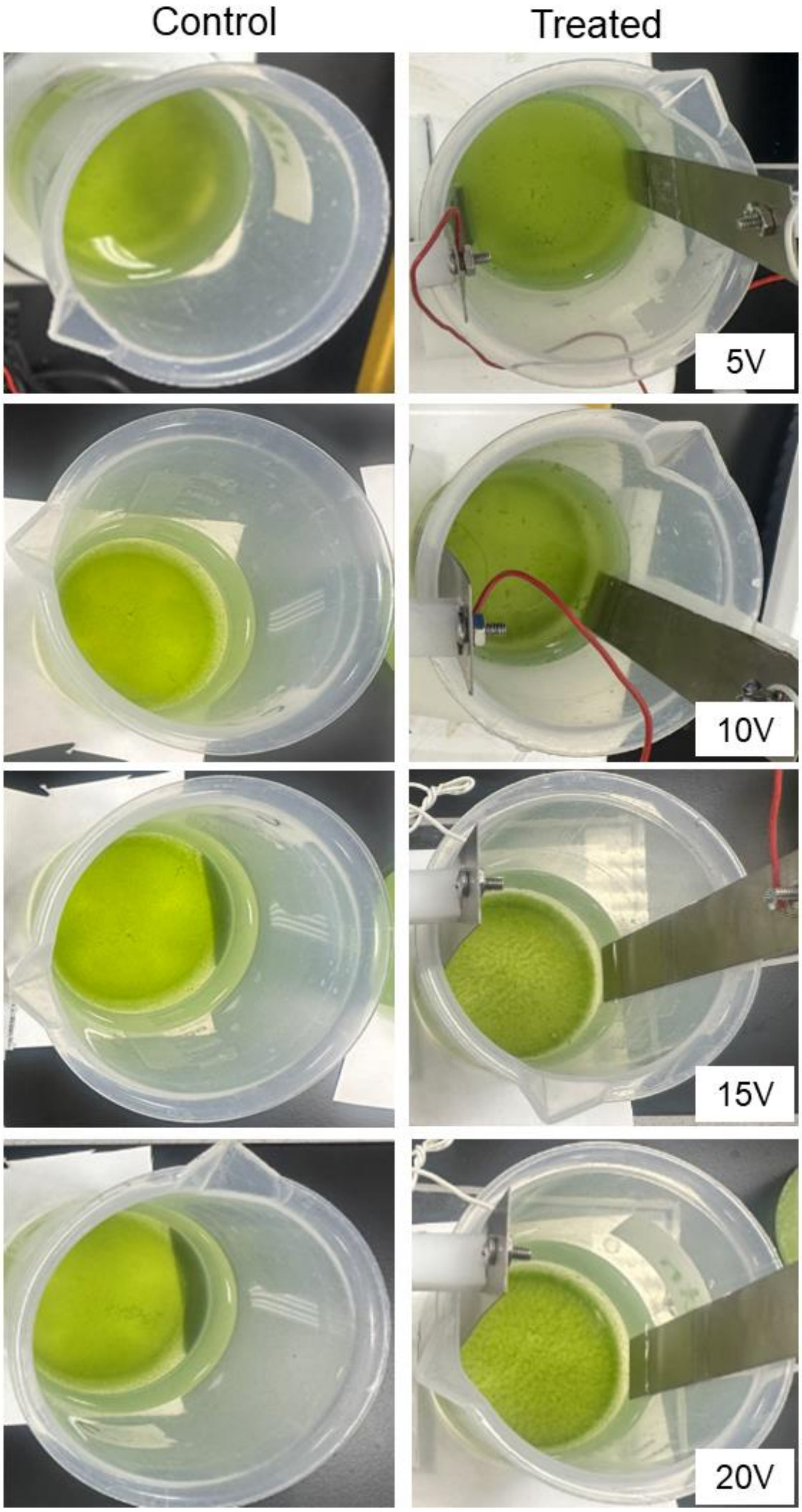


Figure 4.2. Photos of DC-treated versus control samples. The formation of flocs was seen in the treated samples. No significant bubble formation or floatation was observed in these samples.

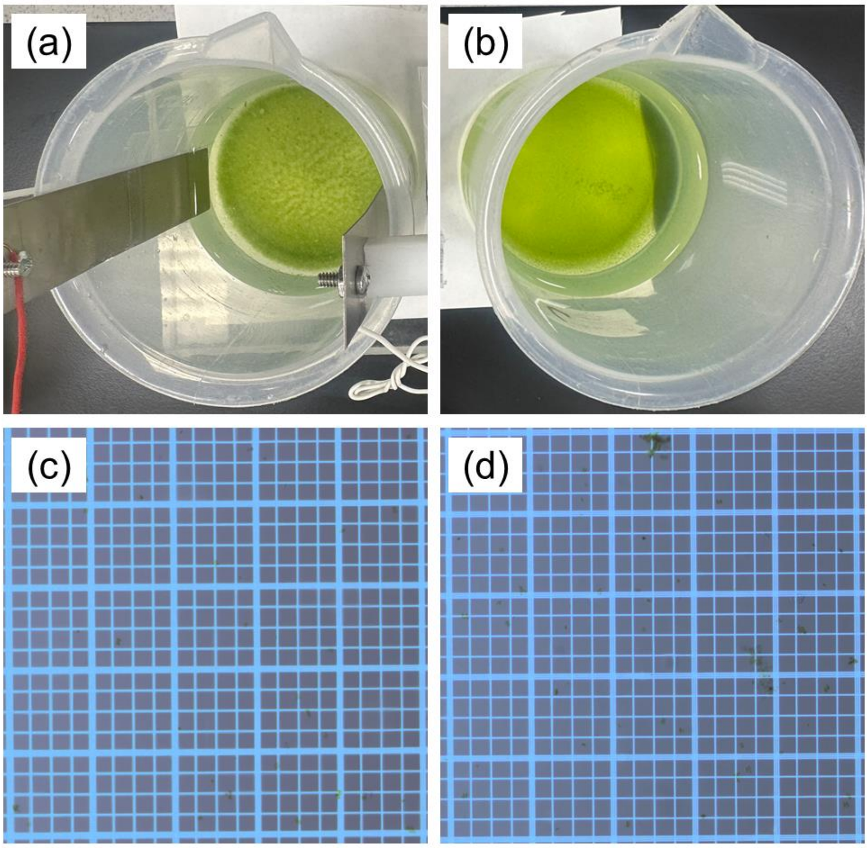


Figure 4.3. (a) Photos of a treated sample (with a voltage of 15 V) versus (b) a control sample (without voltage applied) after jar testing. (c) Microscopic images of supernatant samples of the treated versus (d) the control sample. Algal cell counting r*esults are available in the Supplementary material.*

4.2.3. Algal concentration determination

The determination of DAB concentrations (mg/L) in the supernatants followed the protocol outlined in Osabutey et al. (2023). Flocculation efficiency was calculated as the ratio of the DAB concentrations after versus before flocculation. The optical density of the supernatant was measured using a spectrophotometer at 670 nm (OD_{670}). A calibration curve was established by correlating OD670 readings with DAB concentrations. The cell counts of *Scenedesmus dimorphus* were determined using a Neubauer hemocytometer under an Olympus CX41 LEEDS optical microscope (Olympus Corp., Tokyo, Japan). ImageJ software was employed to facilitate the counting of microalgal cells within hemocytometer squares, with algal cell counts (cells/mL) averaged from nine squares for each sample.

4.2.4. Comparison with chemical flocculation and electrocoagulation

For comparison, chemical flocculation and electrocoagulation experiments were performed. Alum ($Al_2(SO_4)_3$), a prevalent chemical flocculant for water treatment, was added to the microalgal suspension at a dosage of 200 ppm. The experiment was conducted on a jar tester, following the protocol outlined by Haleem et al. (2023). For electrocoagulation, the titanium anode was replaced with an aluminum plate of identical size, and a DC voltage of 15V (174.4/m) was applied for 60 min. Microalgal sediments collected from the aforementioned flocculation experiments were centrifuged to remove excess water before being digested for aluminum analysis, aimed at confirming the potential purity benefits of our proposed method. The digestion process followed a similar protocol described by (Memić, Mahić, Žero, & Muhić-Šarac, 2014). In brief, approximately 3.5 g of wet algal biomass was weighed and transferred into a 50 mL centrifuge tube. A 6-mL mixture of 70% HNO_3 and 37% HCl (in a 2:1 ratio) was added to each tube, followed by the addition of 1 mL of 30% H_2O_2. Each sample was digested for 24 hr and then heated at 95°C for 1 hr. After cooling to room temperature, deionized water was added to the digested sample to bring the volume up to 25 mL. The samples were sent to a commercial analytical lab for Al analysis using a Thermo Scientific iCAP Pro XPS ICP-OES (Thermo Fisher Scientific Inc., Waltham, MA). The content of Al in microalgae was expressed as ng Al per mg of wet algal biomass (ng/mg or ppm).

4.2.5. Zeta potential analysis

The zeta potential (ζ; mV) of the microalgal cells was determined using a Litesizer 500 Zetasizer (Anton Paar, Ashland, Virginia) at a pH of 6.7, which is representative of the BBM, and no pH adjustment was applied. Before the analysis, the suspension was diluted with deionized water to raise transmittance to >85%. The measurement results were averaged from triplicate analyses.

4.3. Results and discussion

4.3.1. Effects of voltage and energizing time

The flocculation efficiency increased with the voltage applied across the titanium electrodes. After 60-min of DC treatment (energization), the efficiency rose from 69.4±0.2% at 5V to 94.3±0.3% at 20V (Table 4.1). All the values were greater than the DAB removal in control samples (no DC voltage applied), indicating elevated microalgal flocculation with the application of DC. For colloidal suspensions, a higher DC electric field leads to enhanced polarization of the EDL (i.e., reorganization of surface charges) of colloidal particles, resulting in greater induced dipole interactions (Shih et al., 2018). The polarization, influenced by charged particle orientation and electric field strength, primarily occurs within an EDL’s diffuse layer (Shih et al., 2015), which includes the slip plane where a zeta potential is defined. The applied DC electric field must be strong enough to overcome initial electric potentials in the diffuse layer (which are tied to the zeta potential) to induce dipole formation. We speculate that a similar explanation applies to microalgal suspensions despite their greater size than colloids.

Table 4.1. Flocculation efficiency at varying voltages and energizing times.

DC voltage (V)	Treatment time (min)	DRB reduction (%)
5	20	31.0±0.1
	40	65.1±0.4
	60	69.4±0.2
	120	66.5±1.4
	Control*	53.1±11.3
10	20	46.9±0.4
	40	55.4±0.4
	60	70.3±0.5
	120	77.6±0.9
	Control*	41.8±3.3
15	20	68.4±0.3
	40	76.0±0.1
	60	86.6±0.3
	120	84.9±1.7
	Control*	53.4±9.6
20	20	83.4±0.7
	40	90.5±0.3
	60	94.3±0.3
	120	92.1±0.4
	Control*	52.4±10.4

Notably, the control samples exhibited considerable microalgal removal due to gravitational settling. This is likely attributed to the relatively large cell size of *Scenedesmus dimorphus* (approximately 4 µm × 10 µm as determined by microscopic analysis) and its tendency to occur as aggregates of four cells. Another potential reason is the microalgae's small zeta potential (-6±1 mV at pH=6.7) leading to a weak repulsive electrostatic force between cell aggregates. For comparison, the zeta potential of *Chlorella vulgaris* was around -18 mV at pH=7.0, according to Procházková et al. (2012).

The flocculation efficiency also increased as the energizing time rose from 20 to 60 min (Table 1). The same trend was affirmed through microscopic counting of microalgal cells (Table 4.2 and Fig. 4.4). Further elongating the energizing time to 120 min, however, resulted in a slight decrease in the flocculation efficiency for the DC=5V, 15V, and 20V scenarios. This is possibly attributed to cell electroporation during elongated electric field exposure, as noted by Joannes et al. (Joannes, Sipaut, Dayou, Yasir, & Mansa, 2015). To mitigate the risk of cell lysis, we decided not to exceed DC=20V and t=60 min for further experiments, following the guidance offered by a previous study on electroporation or electro-permeabilization (Goettel, Eing, Gusbeth, Straessner, & Frey, 2013).

Table 4.2. Algal cells count in the supernatants of control versus treatment samples.

DC voltage (V)	Treatment time (min)	Supernatant algal cell counts (cells /cm^3)
5	20	5.0×10^6
	40	4.8×10^6
	60	3.3×10^6
	Control*	7.4×10^6
10	20	5.9×10^6
	40	4.6×10^6
	60	3.6×10^6
	Control*	7.9×10^6
15	20	5.5×10^6
	40	2.1×10^6
	60	1.2×10^6
	Control*	8.4×10^6
20	20	3.0×10^6
	40	6.0×10^5
	60	4.0×10^5
	Control*	8.2×10^6

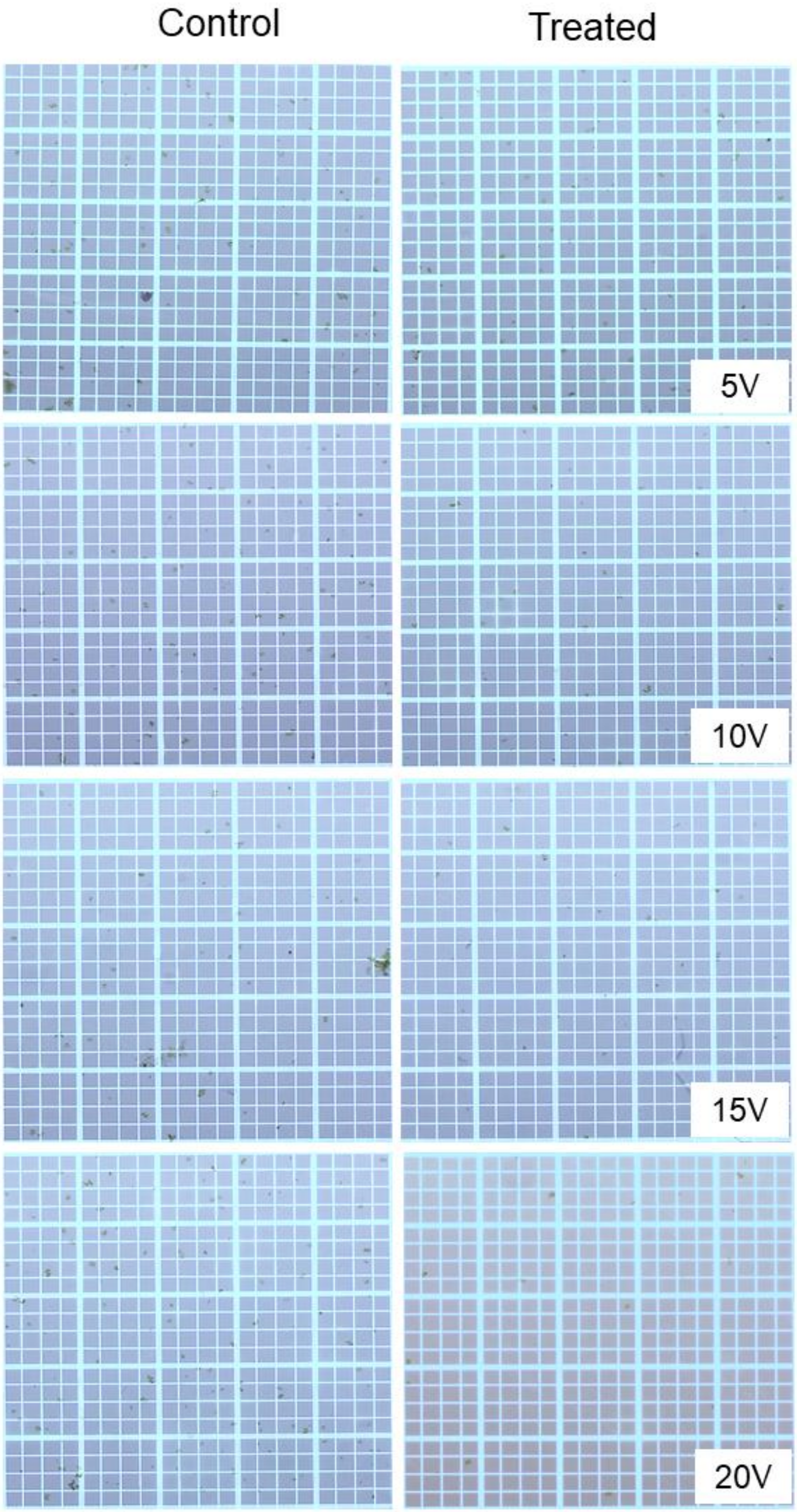


Figure 4.4. Microscopic images of DC-treated versus control samples. Supernatants from these samples were subjected to microscopic algal cell counting. Significantly more algal cells were observed in the control samples.

Both the treatment and control samples were continuously stirred during the energizing time. Stirring is aimed to hydrodynamically facilitate collisions among microalgal cells, and it is also an essential step for flocculation testing using a jar tester. Without stirring, the DAB removal in the control samples was lower by approximately 5 to 10% (Fig. 4.5). Stirring could play a crucial role in DC-initiated flocculation by enabling “irreversible” collisions between two dipoles and the reorganization of surface charges on the formed aggregates, as demonstrated in Mori et al. (Mori et al., 2019).

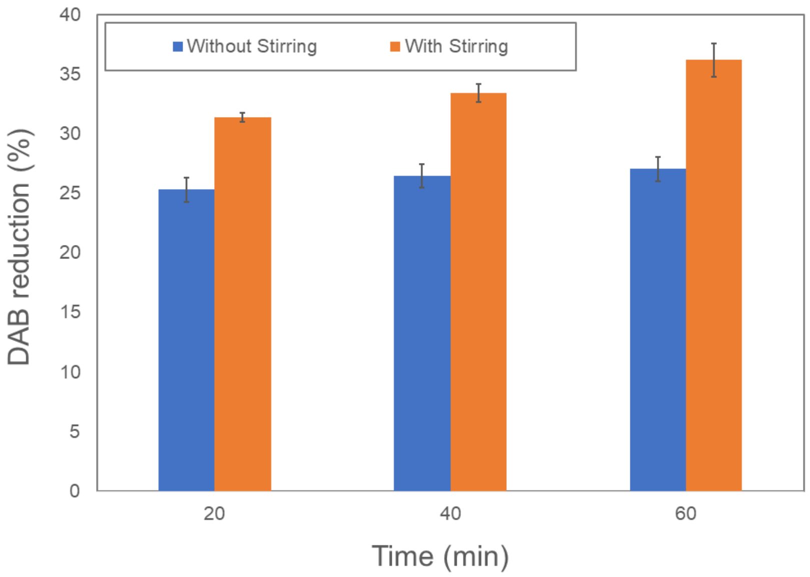


Figure 4.5. DAB reduction due to gravitational settling when no voltage was applied: with versus without stirring.

4.3.2. Comparison with chemical flocculation and electrocoagulation

Three scenarios were compared: (1) chemical flocculation with 200 ppm Alum, (2) electrocoagulation with an aluminum electrode and DC=15V, and (3) DC-initiated flocculation with DC=15V (173 V/m), focusing on energy consumption and impurity levels. An average flocculation efficiency of 77.4%, 83.7%, and 71.7% was observed in the three scenarios, respectively. Despite the superior flocculation performance of electrocoagulation compared to the other two methods, it consumed significantly more electricity (~40 mA) than DC-initiated flocculation (~10 mA). As a side result, the electrocoagulation experiments generated considerably more gas (hydrogen) bubbles compared to DC-initiated flocculation (due to the high overpotential of oxygen and hydrogen evolution reactions on titanium electrodes). Few algal cells were indeed separated through floatation in the DC-initiated flocculation experiments, as illustrated in Fig. 2. Furthermore, electrocoagulation resulted in the highest aluminum content (4.16 ppm) in obtained algal biomass, followed by chemical flocculation (0.93 ppm). A trace amount of aluminum (0.17 ppm) was found in the algal sample obtained from the DC-initiated flocculation experiments, likely originating from aluminum impurities in the BBM or titanium electrodes. Nevertheless, DC-initiation flocculation demonstrated great potential in minimizing impurities in harvested algal biomass.

4.3.3. Discussion

Microalgae typically bear negative surface charges due to functional groups like carboxylic (-COOH) and amino (-NH2) on their cytomembranes, which are vital for cell viability and adherence (N. Li et al., 2022). The interaction of these charges with counter-ions and co-ions in the surroundings lead to the formation of an EDL, which consists of a densely packed, rigid Stern layer and a loosely packed diffuse layer. Zeta potential characterizes the surface potential of a charged particle at the slip plane situating within the diffuse layer, and it is a key factor governing the stability of particle suspensions (J. Zhang, Song, & Li, 2018).

As aforementioned, DC-initiated flocculation operates through the polarization of the EDL (i.e., surface charge reorganization), leading to the formation of induced dipoles when a DC electric field is strong enough (Shilov et al., 2000). These dipoles align themselves in response to the reorganized surface charges. In a DC electric field, particles with dipole moments tend to align with field lines due to electrical torque (Baker-Jarvis & Kim, 2012). When these particles align head-to-tail, they experience attractive forces, as the partially positive charge on one particle is drawn towards the partially negative charge on another particle; whereas when the particles stay head-to-head parallel, repulsive forces occur (Fig. 4.6) (Shih et al., 2018). Additionally, the DC electric field can alter the behavior of charged particles, causing changes in electrophoretic mobility and zeta potential (Velev, Gangwal, & Petsev, 2009).

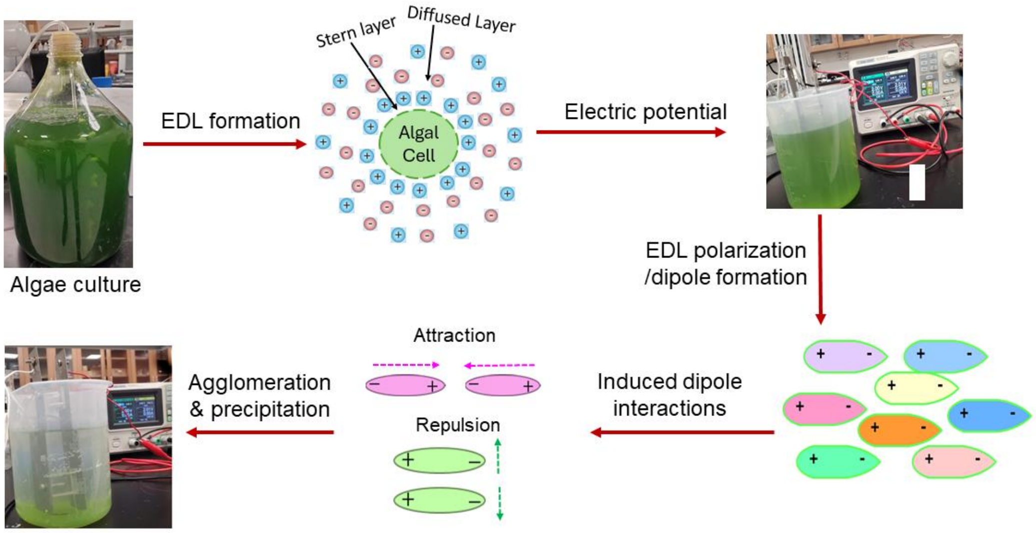


Figure. 4.6. A possible mechanism of DC-initiated microalgae flocculation.

Therefore, the governing mechanism of DC-initiated flocculation is distinct from the four well-known coagulation-flocculation processes, namely charge neutralization, sweep coagulation, bridging, and patch flocculation. Adding Alum or using electrocoagulation with aluminum electrodes both create large aluminum hydroxide ($Al (OH)_3$) precipitates in nearly neutral pH conditions. Microalgal cells become entrapped in these precipitates and settle alongside them through sweep coagulation. Consequently, the resulting microalgal sediments from these two experiments exhibited a different feature (large, loose flocs) than DC-initiated flocculation (small, dense flocs).

Moving forward, microalgal species (e.g., *Chlorella vulgaris*) with smaller cell sizes and a greater zeta potential than *Scenedesmus dimorphus* will be tested for DC-initiated flocculation. The investigation will be focused on any potential relationship between zeta potential and the minimum and optimal voltage gradient required for effective flocculation. Additional characterization of microalgal suspensions and electrodes, along with theoretical analyses, will be conducted to elucidate the underlying mechanism. Notably, without the addition of any chemicals, DC-initiated flocculation may be seamlessly interfaced with filtration (e.g., using a stainless-steel sieve as the cathode), reducing operating costs and fouling issues.

4.4. Conclusions

This study demonstrated the viability of DC-initiated flocculation as a simple yet promising method for microalgae harvesting. It systematically investigated the effects of DC voltage and energizing time on the flocculation of *Scenedesmus dimorphus* suspensions. Under the optimal condition (DC=20V and t=60 min), a flocculation efficiency of 94% was obtained. While slightly less efficient compared to chemical flocculation using Alum or electrocoagulation using Al electrodes, DC-initiated flocculation resulted in microalgal biomass with significantly lower Al contents. The flocculation process within a DC electric field was attributed to the polarization of the EDL of microalgal cells and subsequently induced dipole interactions.

CHAPTER 5

CONCLUSIONS & FUTURE DIRECTIONS

5.1. Conclusions

5.1.1. Overview

Coagulation and flocculation are acknowledged as effective and economical methods for eliminating suspended particles from water and wastewater. However, traditional chemical coagulation processes often lead to secondary pollution and can be costly due to chemical usage and time consumption. Consequently, there is a growing interest in developing environmentally friendly and cost-effective natural and plant-based flocculants. Researchers are actively exploring the potential of natural flocculants, such as starch and cellulose, which have been previously investigated for various wastewater treatment applications. In this study, the focus is on developing natural flocculants from potato peels and commercial cellulose. Initially, starch is isolated from potato peel waste and modified to cationic starch, while commercial cellulose is also modified to cationic cellulose. These modified flocculants are then applied in dairy and swine wastewater treatment to remove suspended particles. The application of these natural flocculants extends beyond dairy and swine wastewater treatment, as they are also tested in scenarios involving aqueous methyl orange and algae harvesting. Additionally, electro-flocculation is explored as a method for algae (Scenedesmus dimorphus) harvesting. This process involves inserting an inert electrode (such as titanium) into the algal culture and applying direct current (DC) to polarize the electric double layer of suspended algal cells, which typically carry a negative charge. This polarization results in the formation of dipoles, leading to the aggregation of suspended particles and enhancing the flocculation rate. Overall, this study highlights the potential of natural and modified flocculants derived from potato peels for various wastewater treatment applications and demonstrates the effectiveness of electro-flocculation in algae harvesting. These developments contribute to ongoing efforts to develop sustainable and efficient methods for water treatment and environmental protection.

5.1.2. Chapter 2 conclusion

In the first experimental chapter, Chapter 2, converting potato peel (PPW), an agricultural byproduct into value-added products is explored for enhancing agricultural sustainability. It would be even more beneficial if these products could find agricultural applications, thereby facilitating a circular economy. This study focuses on extracting starch from PPW, an abundant agricultural byproduct, and converting it into cationic starch flocculants through a simple wet chemistry process. The prepared cationic starch samples demonstrated decent performance in flocculating and removing TSS from swine and dairy wastewater. Further enhancement of TSS removal efficiency could be achieved by increasing the DS of cationic starch using different catalysts or solvents. Compared to many commercial flocculants, the prepared cationic starch required relatively low application dosages for the same amount of TSS removal. Since microbes in livestock wastewater are generally negatively charged, efforts will be made to further study the flocculation removal of waterborne coliforms and antibiotic-resistant bacteria. This study is anticipated to stimulate the valorization of agricultural waste and facilitate the development of eco-friendly, innovative solutions for livestock wastewater management.

5.1.3. Chapter 3 conclusion

In the second experimental chapter, Chapter 3, cationic cellulose was synthesized from commercial cellulose through homogeneous etherification with CHPTAC in an alkaline medium. Different cationic cellulose (CC) samples were obtained by adjusting reaction parameters to achieve varying degrees of substitution (DS). The optimal reaction conditions, resulting in a maximized DS, included a 4-hour reaction time (for both alkalization and etherification), 0.124M of CHPTAC, 3M of NaOH, and a reaction temperature of 60°C. Structural analyses using FT-IR, DSC, SEM, and NMR confirmed the successful introduction of quaternary ammonium groups onto the cellulose backbone. One of the prepared CC samples with a DS ranging from 0.845 to 0.865 was evaluated as a flocculant/sorbent in three different application scenarios. It exhibited satisfactory efficiency in capturing and removing algal biomass (up to 93.0%), total suspended solids (up to 53.3%), and methyl orange (up to 53.0%) from microalgal cultural broth, dairy wastewater, and synthetic dye wastewater, respectively. Further optimization of pH and CC dosage levels could potentially enhance the efficiency of these removal processes. This study underscores the significant potential of CC in wastewater treatment applications and is expected to encourage further exploration and development of natural flocculants.

5.1.4. Chapter 4 Conclusion

Chapter 4 of this study demonstrates the effectiveness of DC-initiated flocculation as a straightforward yet promising method for microalgae harvesting. It systematically explores the impact of DC voltage and energizing time on the flocculation of Scenedesmus dimorphus suspensions. Under optimal conditions (DC=20V and t=60 min), a flocculation efficiency of 94% was achieved. While slightly less efficient than chemical flocculation using Alum or electrocoagulation using Al electrodes, DC-initiated flocculation resulted in microalgal biomass with significantly lower aluminum contents. The flocculation process within a DC electric field is attributed to the polarization of the electric double layer (EDL) of microalgal cells, followed by induced dipole interactions.

5.2. Future directions

5.2.1. The future of natural flocculants

The future of natural flocculants is promising in growing environmental concerns about traditional water treatment methods. Traditional approaches, such as using aluminum and iron-based coagulants, face increasing environmental challenges (Kurniawan et al., 2020). While bio-flocculants offer a viable alternative, their development is hindered by the biodegradable nature of their components, resulting in a shorter shelf life (Othmani, Rasteiro, & Khadhraoui, 2020). Overcoming these challenges and providing strategic recommendations are essential for advancing sustainable water treatment. Natural flocculants have the potential to address global water pollution by targeting both insoluble and soluble contaminants in wastewater (El-Gaayda et al., 2021). Developing specific criteria for wastewater management, considering factors like wastewater type and production costs, is crucial for maximizing their effectiveness. Efforts to address challenges associated with natural flocculant composition include developing more efficient materials for monitoring the Degree of substitution (DS) to enhance treatment efficiency.

In the future, exploring natural flocculants for bacteria flocculation shows promise, as bacteria typically carry a negative charge, while modified cellulose and starches possess cationic properties that facilitate aggregation. Investigating the potential of natural flocculants for bacteria aggregation in livestock wastewater could address health issues associated with untreated discharges. For instance, untreated swine wastewater, known for its contamination with salmonella and E. coli (Cordero, García, Herradora, Ramírez, & Martínez, 2010) can contaminate freshwater reservoirs when mixed with rainwater. Additionally, natural flocculants can target both insoluble and soluble contaminants and may be explored for removing chemical oxygen demand (COD) in sugar industry effluent. The long chains of modified starch and cellulose make them suitable for treating freshwater, including addressing challenges such as algal blooms and heavy metal contamination. Establishing criteria for wastewater management, conducting scaled-up studies, and developing efficient materials with a good degree of substitution (DS) are crucial steps in realizing this potential. Additionally, improving public awareness and securing governmental support is essential for the widespread adoption of natural flocculants in water treatment practices.

5.2.2. Future of electro-flocculation

The outlook for electro-flocculation in agricultural water and wastewater treatment using inert electrodes offers a promising path for addressing the specific challenges in this sector. Unlike traditional chemical methods, electro-flocculation utilizes electric fields generated by inert electrodes to induce flocculation and coagulation, providing benefits for agricultural applications. A key area for future advancement involves optimizing the design and engineering of electro-flocculation systems customized for agricultural contexts. Researchers are exploring innovative configurations and materials for inert electrodes to boost efficiency while reducing energy consumption. By refining electrode designs and power delivery mechanisms, we can achieve higher rates of contaminant removal, such as suspended solids, nutrients, pesticides, and organic matter, thus enhancing water quality for agricultural purposes. Moreover, integrating electro-flocculation with other water treatment technologies like membrane filtration and biological treatment holds promise for improving treatment efficiency and addressing the diverse range of contaminants found in water & wastewater (Sravan, Matsakas, & Sarkar, 2024). Through the combination of electro-flocculation with these complementary processes, we can achieve synergistic effects, leading to enhanced water quality and resource recovery such as efficient phosphate management in livestock wastewater.

Furthermore, the use of inert electrodes in electro-flocculation systems offers advantages such as durability and stability (Alkhadra et al., 2022) making them compatible with a wide range of water chemistries commonly encountered in agricultural settings. This ensures reliable operation and minimal maintenance requirements. As research in this field progresses, there is potential to explore the application of alternating current (AC) in addition to direct current (DC) for electro-flocculation in agricultural contexts. Comparing the efficiencies of AC and DC electro-flocculation systems can provide insights into their respective advantages and suitability for removing both organic and inorganic pollutants commonly found in agricultural water and wastewater. The future of electro-flocculation in agricultural water and wastewater treatment using inert electrodes holds promise, as ongoing research and integration with other treatment techniques may elevate water quality and promote environmental sustainability.

5.2.3. Future directions for harvesting of microalgae.

In recent years, microalgae have gotten significant attention as a promising source of various valuable products, including biofuels, nutraceuticals, pharmaceuticals, and cosmetics. However, traditional methods of microalgae harvesting, holding physical and chemical approaches, encounter challenges related to environmental impact and cost-effectiveness. Chemical methods, notably electrocoagulation, utilize metal anodes to release metal ions that neutralize algal cells and enhance flocculation. However, this process can introduce impurities into the fresh algal biomass, thereby limiting its suitability for industrial applications requiring high purity, such as pharmaceuticals and cosmetics.

In the future, microalgae harvesting is poised for significant advancements aimed at enhancing efficiency, sustainability, and cost-effectiveness. Traditional methods of microalgae harvesting, including both chemical and physical approaches, often raise environmental concerns and may not be economically viable in the long term. Consequently, there is a pressing need for innovative strategies that can overcome these challenges. One promising direction involves the development of novel harvesting technologies that prioritize environmental friendliness and economic feasibility. For example, the utilization of inert electrodes, such as titanium electrodes, emerges as a sustainable alternative capable of reducing the risk of contaminants in harvested microalgae, thus rendering them suitable for various industrial applications.

Additionally, ongoing research efforts focus on optimizing harvesting processes, exploring alternative materials, and integrating advanced technologies to further enhance the efficiency and sustainability of microalgae harvesting. These endeavors hold great promise for revolutionizing the future of microalgae harvesting, with potential benefits spanning industries such as biofuel production, pharmaceuticals, and food supplements.

Our work specifically addresses the challenges associated with microalgae (S. diamorphus) harvesting, emphasizing the need for environmentally friendly and cost-effective methodologies. By introducing inert electrodes, particularly titanium, as a viable alternative, we aim to minimize contaminants in harvested microalgae, thus making them more suitable for industrial applications. While microalgae have garnered significant attention as a valuable source of various products, including biofuels and pharmaceuticals, traditional harvesting methods face obstacles related to environmental impact and cost-effectiveness. Chemical approaches, such as electrocoagulation, while effective in promoting flocculation, may introduce impurities into the harvested biomass, limiting its suitability for certain industrial applications.

Looking ahead, the future of microalgae harvesting relies on the development of methods that maximize efficiency while minimizing impurities. Electro-flocculation emerges as a promising solution, utilizing inert anodes and electric fields to induce the aggregation of microalgae cells, thereby facilitating their separation from the culture medium. Compared to chemical methods, electro-flocculation offers several advantages, including environmental friendliness and scalability. By optimizing process parameters, researchers can tailor electro-flocculation to specific microalgae species and cultivation conditions, thereby enhancing its efficiency and effectiveness. Therefore, electro-flocculation represents a forward-looking approach to microalgae harvesting, offering high efficiency with minimal impurity introduction.

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APPENDIX – A. Journal publications during PhD studies

1st Author

1. Flocculation of livestock wastewater using cationic starch prepared from potato peels. Environmental Science: Water Research & Technology, 2023, 9, 1690-1700 https://doi.org/10.1039/D2EW00794K

2. Preparation of cationic cellulose as a natural flocculant/sorbent and its application in three water treatment scenarios. Water, 2023, 15(11), 2021. https://doi.org/10.3390/w15112021.

3. Viability of artificial rains for air pollution control: Insights from natural rainfall and roadside sprinkling. Atmosphere 2023, 14(12), 1714. https://doi.org/10.3390/atmos14121714

4. Microplastics and associated chemicals in drinking water: A review of their occurrence and human health implications. Science of The Total Environment, 169594. https://doi.org/10.1016/j.scitotenv.2023.169594

5. DC-initiated flocculation of Scenedesmus dimorphus. Under review in journal of Bioresource Technology.

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6. Particulate Matter in Swine Barns: A Comprehensive Review. Atmosphere 2022, 13(3), 490; https://doi.org/10.3390/atmos13030490.

7. Growth of Scenedesmus dimorphus in swine wastewater with versus without solid–liquid separation pretreatment. Bioresource Technology, 128434. https://doi.org/10.1016/j.biortech.2022.128434.

8. Makeup Water Addition Can Affect the Growth of Scenedesmus dimorphus in Photobioreactors. AgriEngineering 2023, 5(2), 982-991. https://doi.org/10.3390/agriengineering5020061.

9. Distribution of airflow and media moisture content across two vertical bed biofilters. AgriEngineering 2022, 4(1), 179-189. https://doi.org/10.3390/agriengineering4010013.

10. Emerging electro-driven technologies for phosphorus enrichment and recovery from wastewater: A review. Water Research. 120699. https://doi.org/10.1016/j.watres.2023.120699.