



Research Article

Valorization of Shrimp Shell Waste into Chitosan for Efficient Green Harvesting of *Chlorella vulgaris* through Physicochemical and Performance Evaluation

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Abstract: Microalgae harvesting remains a major bottleneck in biomass production due to its high energy and operational costs, driving interest in sustainable and low-cost bio-based coagulants. This study aims to evaluate chitosan derived from *Litopenaeus vannamei* shell waste as a green coagulant for harvesting *Chlorella vulgaris*, with emphasis on its physicochemical characteristics and harvesting performance. Chitosan was synthesized through chitin deacetylation and characterized using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) to assess crystallinity and functional groups. The effects of chitosan dosage, medium pH, and contact time on harvesting efficiency and harvesting kinetics were systematically investigated. The synthesized chitosan exhibited a crystallinity index of 73.76% and a degree of deacetylation of 78.97%, indicating coagulation-relevant protonable amino groups. Harvesting efficiencies exceeding 95% were achieved under optimum conditions of 10 mg L⁻¹ dosage, pH 6, and contact time of 10 min. The rapid decrease in optical density at 680 nm was consistent with rapid algal-cell destabilization and aggregation, likely driven by charge neutralization and polymer bridging mechanisms. Kinetic analysis showed that the first-order model best described the harvesting behavior. The FTIR spectra of the harvested biomass showed no significant peak shifts compared with the control, indicating no major alteration of detectable functional groups. These findings demonstrate the feasibility of converting shrimp shell waste into chitosan as a green coagulant for efficient microalgae harvesting and support its potential application in sustainable biomass recovery.

Keywords: Chitosan; *Chlorella vulgaris*; Kinetics; Microalgae harvesting; Shrimp shell waste

1. Introduction

The rapid growth of the global population and economic activities has led to a continuous increase in energy demand (Ogbonna & Nwoba, 2021). Currently, fossil energy sources dominate the global energy mix, contributing more than 80% of the total primary energy consumption (Zhang et al., 2024). The persistent reliance on fossil fuels poses long-term sustainability challenges because these resources are formed through geological processes over millions of years and are therefore classified as non-renewable (Llaverro-Pasquina et al., 2025). This situation highlights the urgent need to develop sustainable and renewable energy sources that do not directly compete with food resources.

Biofuels, including organic materials originating from plants and animals, represent a promising class of renewable energy derived from biomass (Araby & El Araby, 2024). Microalgae have emerged as a next-generation bioenergy feedstock beyond conventional biodiesel and bioethanol derived from terrestrial crops due to their high growth rates, superior biomass productivity, and ability to accumulate energy-rich biomolecules such as lipids, carbohydrates, and proteins. Various bioenergy products, including biodiesel, biohydrogen, and biogas, can be obtained from microalgal biomass (Yasir et al., 2022).

Chlorella vulgaris has attracted considerable attention owing to its broad applicability in nutrition, aquaculture, and environmental biotechnology. This unicellular green microalga is characterized by high protein content, favorable fatty acid profiles, and robust adaptability to varying cultivation conditions (Schneider et al., 2025). In addition to its value as a biomass source, *Chlorella* species have been widely investigated for nutrient uptake and the removal of toxic compounds from wastewater, further supporting their relevance in integrated bioprocesses (Abdelfattah et al., 2023). The biochemical composition and biomass productivity of *Chlorella* are strongly dependent on strain selection and growth conditions, which directly influence downstream processing's technical and economic feasibility (Dutta et al., 2025).

Despite the promising potential of microalgae-based bioprocesses, significant bottlenecks in downstream processing, particularly during biomass harvesting, limit their large-scale implementation. The harvesting stage, especially thickening and dewatering, accounts for approximately 20%–30% of the total production cost of microalgae (Greque de Morais et al., 2023). Various harvesting techniques, including centrifugation, filtration, flotation, sedimentation, and pelletization, have been developed (Vasistha et al., 2021). However, most of these methods are associated with high energy consumption and operational costs, thereby limiting their economic feasibility for commercial-scale applications (Mun et al., 2025).

Coagulation–flocculation is considered a relatively low-energy method for harvesting microalgae because it promotes cell aggregation into larger flocs that can be readily separated from the culture medium (Zhang et al., 2024). However, conventional inorganic coagulants may cause secondary pollution, contaminate harvested biomass, and restrict downstream use. Natural coagulants, including plant-derived materials, microbial bioflocculants, and biopolymers, have been investigated as alternative harvesting agents. Although these materials are generally biodegradable and less likely to introduce secondary contaminants, their performance varies with coagulant type, dosage, and operating conditions.

Chitosan has been widely studied for microalgae harvesting because of its cationic character and ability to promote floc formation through charge neutralization and polymer bridging. Chitosan is a polysaccharide produced by the partial deacetylation of chitin and contains reactive amino and hydroxyl groups that contribute to its biodegradability, biocompatibility, low toxicity, and chemical reactivity (Narudin et al., 2020, 2022). The amino groups of chitosan become protonated under acidic to near-neutral conditions, producing positively charged polymer chains. These protonated amino groups electrostatically interact with the negatively charged surface of microalgal cells, reducing electrostatic repulsion and promoting floc formation through charge neutralization and polymer bridging. These characteristics make chitosan an effective natural coagulant for harvesting microalgae. Chitosan achieves harvesting efficiencies of approximately 98%–99% at relatively low dosages under optimized conditions (Zamri et al., 2023). Chitosan is mainly obtained from crustacean shells through demineralization, deproteinization, and deacetylation (Hossain & Iqbal, 2014). Whiteleg shrimp (*Litopenaeus vannamei*) shells are particularly relevant as a raw material because they are widely generated as seafood-processing industry by-products. Therefore, their conversion into chitosan provides both a functional natural coagulant and an opportunity for shell-waste valorization. Shrimp shell-derived chitosan can achieve high harvesting efficiencies for microalgae under appropriate dosage and mixing conditions (Islam et al., 2023).

Nevertheless, most previous studies have used commercial chitosan or have not systematically assessed the use of locally produced shrimp shell-derived chitosan for harvesting specific

microalgal species, particularly *Chlorella vulgaris*. The combined effects of chitosan dosage and medium pH remain insufficiently evaluated despite their importance in determining flocculation performance. The physicochemical properties of chitosan depend on its raw material and extraction conditions, which may influence harvesting efficiency and process consistency.

Therefore, this study evaluated chitosan extracted from whiteleg shrimp shell waste as a natural coagulant for *Chlorella vulgaris* harvesting. The effects of chitosan dosage and medium pH on harvesting efficiency were systematically investigated to determine suitable operating conditions. This study also examined the potential of shrimp shell-derived chitosan to support a more resource-efficient microalgae harvesting process within a circular bioeconomy framework.

2. Methods

Chlorella vulgaris culture was obtained from UgoPlanktonShop (Purworejo, Indonesia). HCl (analytical grade) and NaOH (analytical grade) were purchased from Merck (Germany). Guillard's f/2 nutrient medium, consisting of a mixture of macro- and micronutrients, was obtained from the Algae Project (Banyuwangi, Indonesia). Chitosan was prepared from whiteleg shrimp (*Litopenaeus vannamei*) shells. Chloroform (CHCl_3 ; analytical grade) and methanol (CH_3OH ; analytical grade) were purchased from Fulltime (China), whereas 70% ethanol ($\text{C}_2\text{H}_5\text{OH}$; technical grade) was obtained commercially. Synthetic sea salt (ASW SALT MIX, Monsterlaut, Indonesia), which contains a mixture of inorganic salts, and reverse-osmosis water (H_2O) supplied by the Advanced Materials Laboratory, Diponegoro University, were used for culture preparation and dilution.

2.1 Photobioreactor Setup and Microalgae Cultivation

Microalgae cultivation was conducted at the Center of Biomass and Renewable Energy, Diponegoro University, Semarang, Indonesia, following the protocol adapted from Hadiyanto et al. (2022) with minor modifications. *Chlorella vulgaris* was cultivated in a laboratory-scale photobioreactor equipped with continuous aeration and artificial illumination (8 W fluorescent lamps, light intensity ≈ 1500 lux) under a 12 h light/12 h dark photoperiod. The initial culture was diluted with RO water at a 1:1 ratio to obtain a working volume of 1 L per photobioreactor. The culture density was maintained at an optical density (OD) above 1.3 during the scale-up. The salinity was adjusted to 30 ppt using synthetic sea salt. Guillard's f/2 nutrients were added weekly at a dosage of 2 mL L^{-1} to support microalgal growth. A schematic of the photobioreactor system is shown in the supplementary materials (Figure S1).

2.2 Preparation and Characterization of Shrimp Shell-Derived Chitosan

2.2.1 Chitosan extraction

Chitosan was extracted from whiteleg shrimp shell waste through successive pre-treatment, demineralization, deproteinization, and deacetylation steps following established protocols (Hossain & Iqbal, 2014). Briefly, shrimp shells were washed, boiled for 15 min, sun-dried for 48 h, ground into powder, oven-dried at 110°C for 15–30 min, and sieved to 100 mesh. The powder was demineralized by treating it with 1 M HCl (solid-to-liquid ratio 1:10, w/v) under stirring (100 rpm) at $\sim 70^\circ\text{C}$ for 1 h, followed by washing to neutral pH and drying at 80°C for 24 h. Deproteinization was performed using 3.5% NaOH (1:10, w/v) at $\sim 65^\circ\text{C}$ for 2 h under stirring, followed by washing and drying. The resulting chitin was converted to chitosan via deacetylation using 60% NaOH (1:20, w/v) at $\sim 100^\circ\text{C}$ for 4 h. The final chitosan product was washed to neutral pH and dried at 65°C for 24 h.

2.2.2 Determination of the deacetylation degree

Potentiometric titration determined the degree of deacetylation (DD) of the synthesized chitosan, following the procedure adapted from Czechowska-Biskup et al. (2012). Approximately

0.05 g of dried chitosan was accurately weighed and dispersed in 25 mL of 0.1 M HCl. The suspension was stirred continuously for 30 min to allow amino group protonation. Subsequently, 25 mL of deionized water was added, and the mixture was stirred until the chitosan sample was completely solubilized. A standardized 0.1 M sodium hydroxide (NaOH) solution was used as the titrant for the titration experiment. The solution was titrated under constant mixing, and the pH was recorded after each incremental addition of NaOH to obtain a potentiometric titration curve. Two inflection points (V_1 and V_2) were identified on the titration curve, corresponding to the neutralization of excess HCl and the deprotonation of protonated amino groups, respectively. The degree of deacetylation was calculated using Eq. (1):

$$\text{Degree of deacetylation (\%)} = 2.03 \times \frac{(V_2 - V_1)}{m + 0.0042 \times (V_2 - V_1)} \quad (1)$$

where $V_2 - V_1$ is the volume difference (mL) between the two deflection points on the titration curve, m is the weight (g) of the chitosan sample, and the constant 2.03 is an empirically derived factor, as described by Czechowska-Biskup et al. (2012). To ensure reproducibility, all titrations were performed in duplicate.

2.2.3 Coagulation-based harvesting of *Chlorella vulgaris*

Microalgae harvesting experiments were conducted at an initial optical density (OD_{680}) of approximately 2.0. For each experiment, 200 mL of microalgal culture was transferred into a 250 mL glass beaker. Chitosan solution was added at predetermined dosages, and the pH of the culture was adjusted to the desired value using 0.1 M HCl or 0.1 M NaOH. Mixing at 200 rpm for 5–15 min initiated coagulation, after which a 10 min quiescent settling period facilitated floc formation and sedimentation. After settling, a 3 mL aliquot was carefully withdrawn at a depth of 3 cm below the liquid surface to minimize the disturbance of the settled flocs. The overall configuration of the coagulation–flocculation setup and sampling position is illustrated in Figure S2. The optical density of the supernatant was then measured to evaluate the harvesting performance.

2.2.4 Evaluation of the harvesting efficiency

Harvesting efficiency was quantified based on the change in optical density before and after coagulation. The optical density (OD) was measured at 680 nm using a UV–Vis spectrophotometer (Spectroquant® Prove 100, Merck KGaA, Germany). The harvesting efficiency (E , %) was calculated as Eq. (2):

$$E (\%) = \frac{OD_i - OD_f}{OD_i} \times 100 \quad (2)$$

where OD_i and OD_f are the optical densities before and after harvesting, respectively.

2.2.5 Biomass characterization

Harvested biomass was dried and analyzed by Fourier transform infrared (FTIR) spectroscopy (PerkinElmer UATR Spectrum Two, USA) in the range of 400–4000 cm^{-1} to assess potential changes in functional groups compared with control biomass obtained by centrifugation (3000 rpm, 15 min) followed by vacuum drying at 40°C for 4 h.

2.2.6 Coagulation-flocculation kinetics

Coagulation kinetics were evaluated by fitting the OD_{680} decay data to the first- and second-order kinetic models. The first-order model is described by Eq. (3), whereas the second-order model is described by Eq. (4).

$$OD(t) = a + b e^{-k \cdot t} \quad (3)$$

$$OD(t) = \frac{OD(0)}{1 + OD(0) k t} \quad (4)$$

where $OD(0)$ is the initial OD at $t = 0$, k is the apparent rate constant, and a and b are constants. The best-fitting model was selected based on R^2 and reduced χ^2 .

3. Results and Discussion

3.1 Characterization of the Chitosan Coagulant

3.1.1 Crystallinity and phase structure of the samples

The XRD patterns of shrimp shell-derived chitosan and commercial chitosan are presented in Figure 1. Both samples exhibited a dominant diffraction feature in the region of $2\theta \approx 20^\circ$, which is characteristic of the semicrystalline organization commonly reported for chitosan. The principal diffraction maximum was observed at $2\theta = 19.85^\circ$ for the synthesized chitosan and at $2\theta = 20.05^\circ$ for the commercial sample. Although the overall profiles were similar, the synthesized chitosan exhibited a sharper and more intense principal reflection, indicating a greater degree of structural ordering in the measured sample.

Using the same intensity-based CrI method for both samples, the synthesized chitosan exhibited a CrI of 73.76%, whereas the commercial chitosan exhibited a lower CrI of 54.56%. This result confirms that both materials retained a semi-crystalline structure but differed in their crystalline organization degree. Similar variability has been reported for chitosan derived from crustaceans. Rasweefali et al. (2021) demonstrated that the deacetylation time significantly altered the physicochemical and solid-state characteristics of shrimp-derived chitosan, although the resulting XRD profiles remained comparable to those of commercial chitosan. Acosta-Ferreira et al. (2020) reported a crystallinity of 38.2% for shrimp-shell-derived chitosan, whereas Matouri et al. (2024) obtained a relative crystallinity of 32.66% using ultrasound and subcritical-water-assisted extraction. These differences demonstrate that the crystalline organization of chitosan is strongly influenced by the biological source and processing conditions rather than being an invariant material property. Therefore, the higher CrI observed for the synthesized chitosan in this study is consistent with previous literature. For example, an autoclave-assisted deacetylation study reported a crystallinity index of 79.9% for synthesized shrimp-shell chitosan compared with 75.9% for a commercial sample, showing that chitosan with crystallinity comparable to or greater than that of commercial material can be produced with appropriately controlled processing conditions (Andoko et al., 2025). Therefore, the higher crystallinity observed here may reflect differences in the deacetylation conditions, hydrogen-bond organization, molecular characteristics, and structural history of the starting material.

The XRD results establish differences in structural organization between the synthesized and commercial chitosan but should not be interpreted as direct evidence that higher crystallinity improves coagulation performance. Chitosan-mediated coagulation is more directly associated with amino group availability and protonation, deacetylation degree, molecular characteristics, and solution conditions. When evaluating the suitability of the synthesized chitosan for *Chlorella vulgaris* harvesting, the XRD results should be interpreted together with the FTIR and degree of deacetylation data.

3.1.2 Functional groups and deacetylation

The Fourier transform infrared spectroscopy (FTIR) spectra of chitin, synthesized chitosan, and commercial chitosan are presented in Figure 2. All samples exhibited a broad absorption band in the $3400\text{--}3200\text{ cm}^{-1}$ region, which was assigned to overlap O–H and N–H stretching

vibrations. The band minima were observed at approximately 3287 cm^{-1} for commercial chitosan, 3361 cm^{-1} for synthesized chitosan, and 3258 cm^{-1} for chitin. Because the O–H and N–H vibrations strongly overlap in this region, these bands were not interpreted independently as evidence of deacetylation. The clearest spectral differences were observed in the amide region. Chitin exhibited distinct absorptions at approximately 1652 and 1621 cm^{-1} , associated with amide I vibrations of N-acetylated units, together with a band at 1553 cm^{-1} attributed to amide II. In comparison, the synthesized chitosan exhibited a much less pronounced absorption near 1650 cm^{-1} and a band at approximately 1586 cm^{-1} , which was mainly attributed to the N–H bending of the primary amino groups. Commercial chitosan showed corresponding bands at approximately 1645 and 1573 cm^{-1} . The reduced prominence of the amide I region in the synthesized sample, together with the appearance of the primary-amine-related band, is consistent with chitin's partial deacetylation and chitosan formation. Similar changes in the amide region have been reported for chitin and chitosan obtained from crustacean shells (Narudin et al., 2020, 2022).

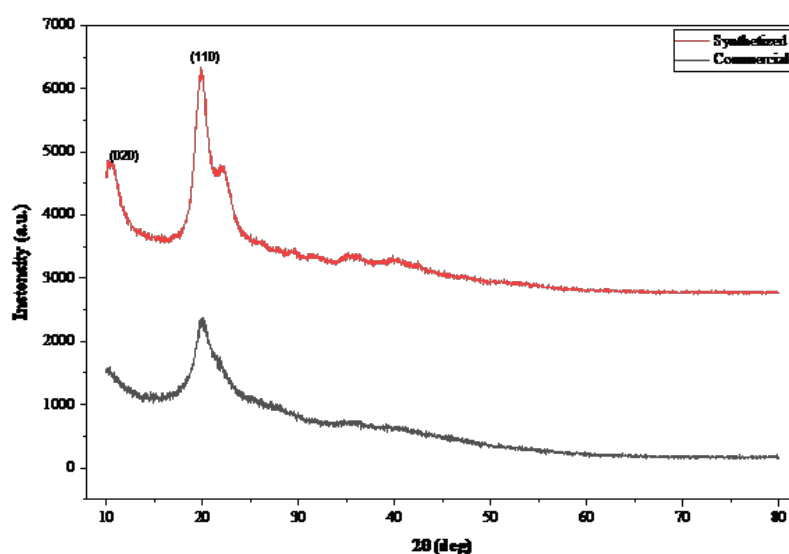


Figure 1 X-ray diffraction patterns of commercial chitosan and shrimp shell-derived chitosan

The synthesized chitosan exhibited bands at 1421 and 1377 cm^{-1} , corresponding to CH_2 bending and CH_3 deformation, respectively. The absorption at 1149 cm^{-1} was assigned to the asymmetric C–O–C stretching vibration of the glycosidic linkage, whereas the bands at 1077 and 1030 cm^{-1} were attributed to the C–O stretching vibrations of the polysaccharide backbone. The band at 893 cm^{-1} corresponds to the β -(1→4)-glycosidic linkage characteristic of chitosan and chitin (Narudin et al., 2020, 2022). The spectral pattern of the synthesized chitosan was comparable to that of commercial chitosan, particularly in the amine and polysaccharide fingerprint regions. Differences in band position and relative intensity may reflect variations in residual acetylation, hydrogen-bonding environment, source material, and sample preparation.

3.1.3 Determination of the degree of deacetylation

The deacetylation degree of the synthesized chitosan was determined by potentiometric titration (Figure 3). The titration curves obtained from two independent runs showed consistent pH transitions during the addition of incremental NaOH, indicating a reproducible neutralization behavior. The calculated deacetylation degree was $78.97\% \pm 2.28$. This value falls within the typical range reported for commercially available chitosan materials.

The degree of deacetylation is a critical parameter that influences the functional performance of chitosan in aqueous systems. A higher degree of deacetylation corresponds to a greater

proportion of free amino groups along the polymer backbone, which can be protonated under mildly acidic to neutral conditions, thereby increasing the polymer's cationic character. The synthesized chitosan exhibited a deacetylation degree of approximately 79%, which is comparable to the values commonly reported for commercial chitosan (Li et al., 2013). This level of deacetylation indicates that protonable amino groups are readily available to facilitate electrostatic interaction with negatively charged microalgal cell surfaces. In coagulation–flocculation systems, amino group density contributes to charge neutralization and may enhance intercellular bridging through polymer chain extension in solution. Therefore, the measured degree of deacetylation provides a chemical basis for the observed harvesting efficiency under optimized conditions. Although coagulation performance is influenced by multiple factors, including crystallinity and operational parameters, the functional group density indicated by the degree of deacetylation likely plays an important supporting role in promoting stable floc formation during *Chlorella vulgaris* harvesting.

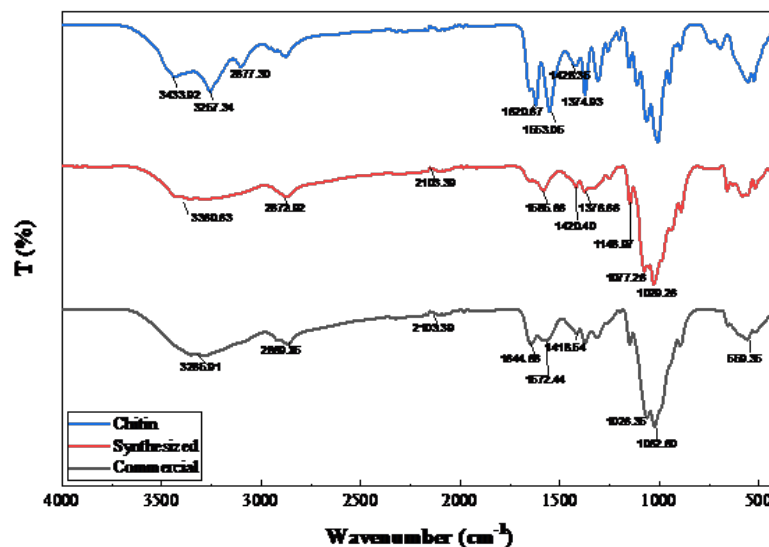


Figure 2 Fourier-transform infrared spectra of chitin, shrimp shell-derived chitosan, and commercial chitosan

3.2 Harvesting Microalgae

Microalgae harvesting is a critical downstream step that largely determines biomass recovery efficiency and overall process feasibility. *Chlorella vulgaris* was harvested via chitosan-assisted coagulation, and the effects of key operational parameters, namely coagulant dosage, medium pH, and coagulation time, were systematically evaluated to identify optimal harvesting conditions. In addition, postharvest biomass characteristics were examined to assess the potential impacts of the coagulation process on biomass quality. This multi-parameter assessment provides a practical basis for optimizing chitosan-based harvesting strategies for scalable microalgae recovery.

3.2.1 Effect of the coagulant dosage

The harvesting efficiency of *Chlorella vulgaris* exhibited a pronounced dependence on chitosan dosage (Figure 4), highlighting the critical role of coagulant concentration in governing flocculation performance. Harvesting efficiency remained relatively limited at low chitosan dosages, which can be attributed to insufficient charge neutralization and inadequate polymer-bridging interactions to destabilize the negatively charged microalgal cells. Under such conditions, only

partial aggregation occurs, resulting in smaller and less stable flocs that are difficult to separate from the culture medium.

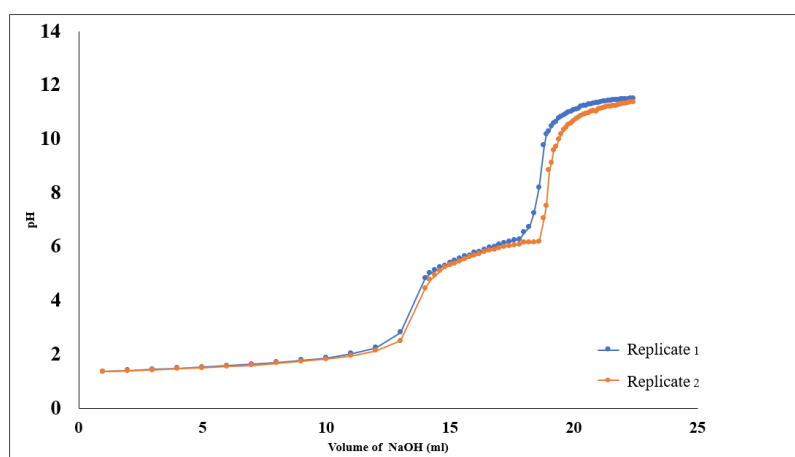


Figure 3 Potentiometric titration curves of chitosan for determining the degree of deacetylation. The pH is plotted as a function of the volume of added NaOH. Replicates 1 and 2 represent two independent titration measurements performed under identical experimental conditions to assess measurement reproducibility

As the chitosan dosage increased, harvesting efficiency improved markedly and reached a maximum at 10 mg L^{-1} , indicating an optimal coagulant concentration. The cationic amino groups of chitosan effectively neutralize the negative surface charges of microalgal cells at this dosage, thereby reducing electrostatic repulsion and facilitating close cell–cell contact. Simultaneously, the polymer chains provide sufficient bridging sites to link multiple cells into larger and denser flocs. The synergistic contribution of charge neutralization and polymer bridging at the optimal dosage results in the formation of compact and settleable flocs, leading to the highest observed harvesting efficiency.

The interaction mechanisms between chitosan and microalgal cells help explain the observed dosage-dependent behavior. At low chitosan dosages, the density of cationic amino groups is insufficient to fully neutralize the negatively charged surface of *Chlorella vulgaris*, resulting in incomplete destabilization and limited floc formation, similar to chitosan and other cationic polymers in microalgal systems (Lama et al., 2016). At 10 mg L^{-1} , the dosage appears sufficient to promote effective charge neutralization while simultaneously enabling polymer-bridging interactions, a synergistic mechanism widely described for cationic biopolymers. At supra-optimal dosages, the efficiency decreased, which is consistent with previous reports that attribute the reduced flocculation performance to charge reversal and polymer-induced restabilization of algal cells and steric hindrance at high polymer coverage.

3.2.2 Effect of pH on harvesting efficiency

The effect of medium pH on the harvesting efficiency of *Chlorella vulgaris* using chitosan as a coagulant is presented in Figure 5. Harvesting efficiency exhibited a clear dependence on pH, with a maximum observed at pH 6. A gradual decline in harvesting efficiency was observed at pH values below or above 6. The reduction in efficiency became more pronounced under alkaline conditions than under mildly acidic conditions. These results indicate an optimal pH range for chitosan-assisted coagulation of *Chlorella vulgaris* under the tested conditions.

The observed dependence of harvesting efficiency on pH can be explained by the chitosan protonation behavior and microalgal cell surface charge characteristics. Chitosan contains primary amine groups with a pKa of approximately 6, meaning that around this pH, a significant fraction of the amino groups exists in the protonated form ($-\text{NH}_3^+$) (Pinho et al., 2024). This pro-

tonation confers a positive charge to the polymer chains, enabling electrostatic interaction with negatively charged functional groups present on the surface of *Chlorella vulgaris* cells (Pinho et al., 2024). At pH 6, the balance between protonation and polymer stability is favorable for charge neutralization and polymer-bridging interactions. Chitosan chains remain sufficiently soluble and flexible to effectively interact with multiple cells, promoting the formation of larger and more compact flocs. Such aggregation enhances sedimentation behavior and increases harvesting efficiency.

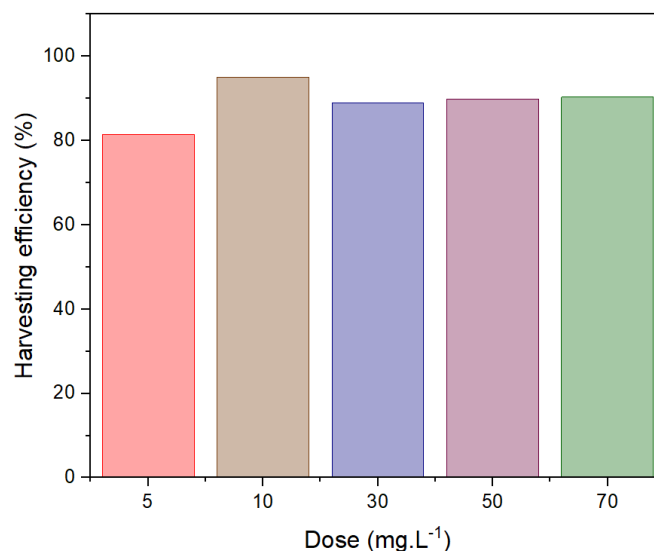


Figure 4 Effect of shrimp shell-derived chitosan dosage on *Chlorella vulgaris* harvesting efficiency at constant pH and coagulation time

At lower pH values, excessive protonation may increase chitosan solubility and reduce its ability to function as an effective bridging agent, as highly soluble chains may remain dispersed rather than forming intercellular networks. Conversely, deprotonation of amino groups reduces the positive charge density of chitosan at alkaline pH, weakening electrostatic attraction with negatively charged microalgal cells (Labeeuw et al., 2021). In addition, increased negative surface charge on the cells may enhance electrostatic repulsion, thereby limiting aggregation. Therefore, the decline in harvesting efficiency outside pH 6 can be attributed to changes in the protonation equilibrium that affect both the polymer charge density and interparticle interactions. These findings highlight the importance of pH control to maintain optimal charge balance in chitosan-mediated microalgae harvesting systems.

3.2.3 Effect of the coagulation time

The harvesting efficiency of *Chlorella vulgaris* varied as a function of coagulation time (Figure 6). At 5 min, the efficiency was comparatively lower. Increasing the coagulation time to 10 min resulted in the highest efficiency observed under the tested conditions. A slight decline in harvesting efficiency was detected at 12.5 and 15 min beyond 10 min. Although the reduction was not drastic, the efficiency did not further improve with an extended coagulation time. These results indicate an optimal coagulation time for chitosan-assisted harvesting under the selected operating conditions.

The time-dependent behavior observed in Figure 6 reflects the dynamic balance between floc formation and floc breakage during chitosan-mediated coagulation. At short contact times (5 min), the limited interaction between destabilized microalgal cells restricts aggregate growth. Under these conditions, charge neutralization and polymer-bridging processes are initiated but may not have progressed sufficiently to form compact and settleable flocs. The maximum efficiency at 10 min indicates that this duration provides adequate time for effective collision

frequency, charge neutralization, and intercellular bridging to occur simultaneously. Similar time-dependent optimization behavior has been reported in chitosan-based microalgae harvesting systems, where floc consolidation requires sufficient contact time to reach structural stability (Vandamme et al., 2012, 2014; Xu et al., 2013). The slight decline observed at prolonged coagulation times (≥ 12.5 min) may be attributed to floc restructuring or breakage under continuous mixing. Extended agitation increases inter-floc collisions and mechanical stress, which can fragment previously formed aggregates into smaller, less readily separated particles. Partial redispersion of loosely bound cells may also contribute to reduced net efficiency (Kumar et al., 2022). From a process optimization perspective, the identification of an optimal coagulation time highlights the importance of balancing floc growth and mechanical stability. Extending the coagulation time beyond the optimum does not enhance the performance and may increase the energy input without additional benefit. Therefore, precise control of contact time is essential for efficient and energy-conscious harvesting of microalgae.

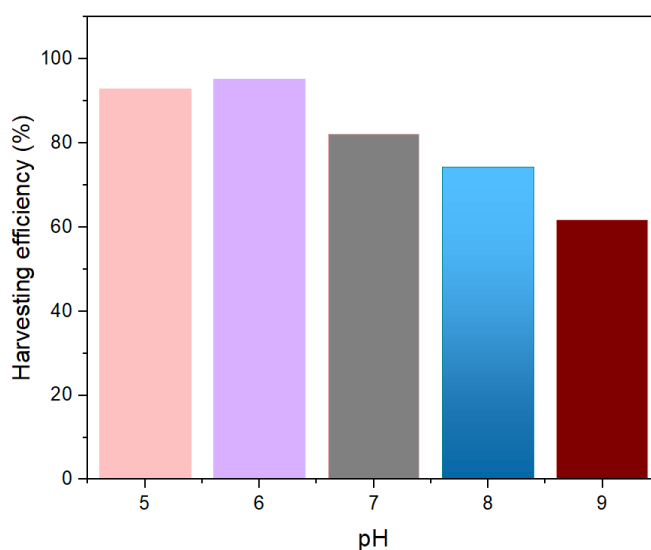


Figure 5 Effect of medium pH on the harvesting efficiency of *Chlorella vulgaris* using chitosan derived from shrimp shells (10 mg L^{-1} , 10 min contact time)

3.3 Post-Harvest Biomass Characterization

The Fourier-transform infrared (FTIR) spectra of *Chlorella vulgaris* biomass harvested via chitosan-assisted coagulation and untreated control biomass are shown in Figure 7. Both spectra exhibited comparable absorption profiles across the wavenumber range of $4000\text{--}500 \text{ cm}^{-1}$. After coagulation, no additional absorption bands were observed, and no noticeable peak shifts were detected in the main characteristic regions. The broad band at $3200\text{--}3400 \text{ cm}^{-1}$ was present in both samples and corresponds to overlapping O–H and N–H stretching vibrations. Prominent bands at approximately 1650 cm^{-1} and 1540 cm^{-1} , assigned to amide I (C=O stretching) and amide II (N–H bending) vibrations, respectively, were observed (Acosta-Ferreira et al., 2020). In addition, the region between $1200\text{--}1000 \text{ cm}^{-1}$ displayed characteristic absorptions associated with C–O–C and C–O stretching of polysaccharides. Although minor differences in band intensity were noted, particularly in the amide region ($1600\text{--}1500 \text{ cm}^{-1}$), the overall spectral patterns of harvested and control biomass remained highly similar.

The similarity between the FTIR spectra of harvested and untreated biomass suggests that chitosan-assisted coagulation did not induce detectable alterations in the primary functional groups of *Chlorella vulgaris* under the applied conditions. The absence of new absorption bands and significant peak shifts in the main protein- and polysaccharide-associated regions is

consistent with previous observations that chitosan mainly interacts with microalgal cells via surface adsorption and charge neutralization rather than via covalent modification of intracellular biomolecules (Renault et al., 2009). Slight variations in band intensity in the amide region can reasonably be attributed to residual surface-bound chitosan, whose amine and amide groups also contribute to absorption in this wavenumber range, rather than to cellular component structural transformation. Similar preservation of the overall FTIR fingerprint of microalgal biomass after chitosan-mediated harvesting has been reported for *Chlorella* and other species, where no new bands and only minor intensity changes were detected following flocculation, indicating that the biochemical composition of the biomass remained largely intact (Zou & Xu, 2024). These findings support a coagulation mechanism dominated by electrostatic interactions between protonated amino groups on chitosan and negatively charged sites on the cell surface, complemented by polymer-bridging effects, as widely described for chitosan-based flocculation systems (Renault et al., 2009). From a bioprocessing perspective, the retention of characteristic protein- and polysaccharide-related FTIR bands is advantageous, as it suggests that the general chemical fingerprint of the biomass is maintained, which is favorable for downstream valorization routes such as lipid extraction, protein recovery, or broader biorefinery applications. Nonetheless, more detailed compositional or targeted biochemical analyses (e.g., lipid class profiling, amino acid analysis, or pigment quantification) would be required to conclusively rule out subtle changes that may not be captured by FTIR alone.

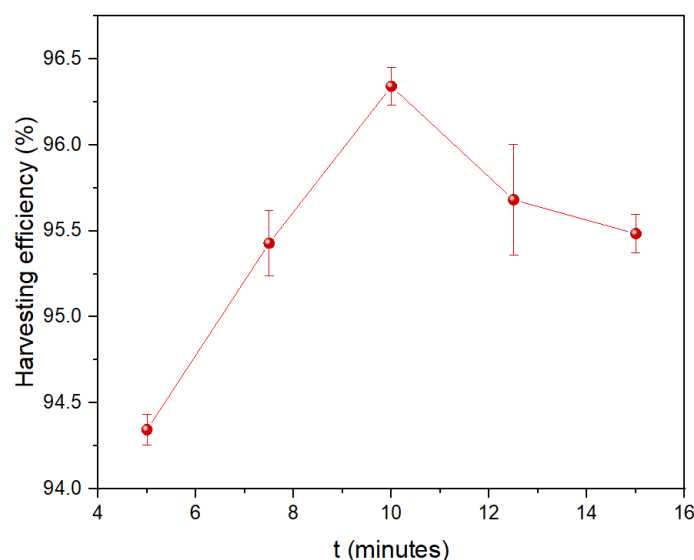


Figure 6 Dependence of *Chlorella vulgaris* harvesting efficiency on coagulation time under optimized chitosan dosage and constant pH conditions

3.4 Coagulation Kinetics

Figure 8 illustrates the temporal evolution of the microalgal culture optical density OD_{680} during coagulation–flocculation. A sharp decline was observed in the initial stage, with OD_{680} decreasing from 1.246 at $t = 0$ min to 0.143 at $t = 1$ min, followed by a slower decrease that approached a residual OD at longer contact times. These results indicate that clarification predominantly occurred during the early phase of the process. To quantitatively describe the OD_{680} decay profile, the experimental data were fitted using first-order and second-order kinetic models (Figure 8; Table S1). The first-order model provided the best agreement with the experimental data, as indicated by $R^2 = 0.999$ and a reduced $\chi^2 = 1.37 \times 10^{-4}$, yielding a rate constant of $k_1 = 2.503 \pm 0.126 \text{ min}^{-1}$. The second-order model also showed a good fit, although slightly inferior ($R^2 = 0.99788$; reduced $\chi^2 = 3.03 \times 10^{-4}$), with $k_2 = 5.338 \pm 0.493 \text{ OD}^{-1}\text{min}^{-1}$.

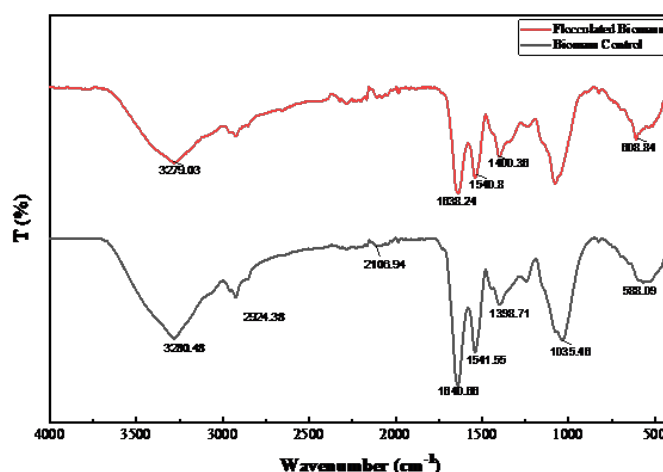


Figure 7 FTIR spectra of *Chlorella vulgaris* biomass harvested via chitosan-assisted coagulation compared with untreated control biomass

The very sharp decrease in OD₆₈₀ within the first minute indicates that rapid coagulation and flocculation occurred during the early stage. This two-regime pattern, characterized by an initial rapid decline followed by a slower tailing phase, is frequently reported in the harvesting of microalgae. The early stage is typically governed by rapid cell surface destabilization and the formation of larger flocs, whereas the subsequent stage is dominated by floc restructuring, finer flocs, or a more stable suspended fraction that cannot be completely removed by gravitational settling alone. A similar trend has been reported for the chitosan-based flocculation of *Chlorella*, where floc formation occurs rapidly at the beginning and then slows as the system approaches a plateau. Based on the goodness-of-fit metrics and the physical relevance of a residual OD baseline, the first-order model was selected as the primary representation of the OD₆₈₀ decrease. The second-order model also showed good agreement with the experimental profile and supports the contribution of particle-to-particle interactions to aggregation dynamics.

Microalgal cells tend to carry a negative surface charge, which stabilizes them in suspension. Chitosan, particularly when its amino groups are protonated, can promote aggregation through two main mechanisms: charge neutralization, which reduces electrostatic repulsion, and polymer bridging, in which polymer chains connect multiple cells into larger aggregates (Zhu et al., 2021). Previous studies on the coagulation and flocculation of *Chlorella* using chitosan have also reported a rapid initial process and rate constants on the order of min⁻¹, supporting that chitosan-based systems can exhibit very fast early-stage kinetics, consistent with the OD₆₈₀ profile observed in this study (Hadiyanto et al., 2022). Moreover, evaluations of chitosan flocculants across multiple algal species, from laboratory to pilot scale, indicate that chitosan performance depends on growth conditions and medium characteristics. Therefore, the tailing behavior and residual OD observed at later times should be interpreted as part of the aggregation dynamics and floc stability, rather than as evidence of process failure.

3.5 Proposed Coagulation-Flocculation Mechanism

Based on the harvesting, kinetic, and FTIR results, the coagulation–flocculation of *Chlorella vulgaris* by chitosan is proposed to occur through initial electrostatic destabilization followed by cell aggregation. Under mildly acidic conditions, the amino groups of chitosan are protonated, increasing the polymer’s positive charge density. These protonated groups can interact with negatively charged sites on the microalgal cell surface, thereby reducing electrostatic repulsion between cells and promoting destabilization. The marked decrease in OD₆₈₀ during the first minute indicates that cell destabilization and aggregation occurred rapidly during the initial

treatment stage. Chitosan chains may interact with more than one microalgal cell after adsorption onto the cell surface, thereby promoting polymer bridging and the formation of larger, settleable flocs. The subsequent slower decrease in OD₆₈₀ may reflect the settling and restructuring of the remaining smaller aggregates in suspension. The relative contribution of charge neutralization and polymer bridging is expected to depend on chitosan dosage, pH, microalgal species, and culture medium composition.

The FTIR spectra of the harvested biomass showed no new absorption bands or substantial peak shifts compared with the untreated biomass. Although this finding does not directly confirm a specific molecular interaction, this finding is consistent with coagulation predominantly involving noncovalent interactions and physical aggregation rather than major chemical modification of the biomass. The proposed mechanism consists of three main steps: protonation of the chitosan amino groups, electrostatic interaction and partial neutralization of the negatively charged cell surface, and subsequent aggregation through polymer bridging. Because zeta potential and floc size measurements were not performed, this mechanism should be interpreted as a plausible explanation supported by the present observations and previous literature rather than as a direct confirmation of a single dominant pathway.

3.6 Comparative Performance of Natural and Bio-Based Coagulants for Harvesting Microalgae

The harvesting performance of the shrimp shell-derived chitosan was compared with selected natural and bio-based coagulants reported in previous studies (Table S2). The comparison is intended to provide a literature-based context rather than a direct ranking because the studies differed in microalgal species, biomass concentration, culture medium, coagulant preparation, dosage basis, mixing conditions, and separation time. Chitosan can provide high harvesting efficiency for freshwater microalgae, particularly under acidic conditions. Rashid et al. (2013) reported that 120 mg L⁻¹ chitosan recovered 92 ± 0.4% of *Chlorella vulgaris* within 3 min, while the maximum recovery of 99 ± 0.5% was obtained at pH 6. Xu et al. (2013) reported more than 99% clarification of *Chlorella sorokiniana* below pH 7 using an optimum chitosan requirement of approximately 10 mg g⁻¹ dry biomass. These results indicate that volumetric concentration alone does not adequately represent chitosan demand, as the required dose is also related to the amount of biomass present in the suspension.

In this study, more than 95% of *Chlorella vulgaris* was harvested using 10 mg L⁻¹ shrimp shell-derived chitosan at pH 6 within 10 min. The applied concentration was lower than that reported by Rashid et al. (2013), but this difference does not necessarily indicate greater intrinsic performance because the initial biomass concentration and experimental procedures were not equivalent. Consistent effectiveness at pH 6 supports the importance of protonation in chitosan-mediated harvesting. Under acidic conditions, amino group protonation increases the positive charge density of chitosan, facilitating its interaction with negatively charged microalgal cell surfaces. The dosage reported by Xu et al. (2013) also highlights an important limitation in cross-study comparison. Their chitosan requirement was normalized to algal dry mass, whereas the present study and several other reports expressed the dosage as mass per unit volume. The volumetric dosage is influenced by the initial biomass concentration and cannot therefore be directly compared with a biomass-normalized dosage. Reporting both units where biomass concentration data are available would provide a more informative basis for evaluating chitosan consumption.

Other natural polymers have achieved similar high harvesting efficiencies under different operating conditions. For example, Yang et al. (2021) reported more than 98% harvesting of *Chlorella vulgaris* using 30 mg L⁻¹ Tanfloc. This result shows that natural cationic polymers other than chitosan can also provide efficient biomass separation. However, the higher reported dosage cannot be interpreted as a lower performance because the flocculant charge density, molecular structure, biomass loading, and experimental procedure differed from those used in the present study. Plant-derived coagulants showed several performances. *Moringa oleifera* extract

achieved 75.5% harvesting efficiency at 8 mg mL⁻¹, whereas its combination with chitosan increased the efficiency to 95.76%. The improvement obtained using the combined formulation is consistent with enhanced biomass aggregation through interactions between different flocculating agents. However, the plant extract concentration was substantially higher than the chitosan concentration used in the present study, and its preparation method represents an additional variable when comparing process performance.

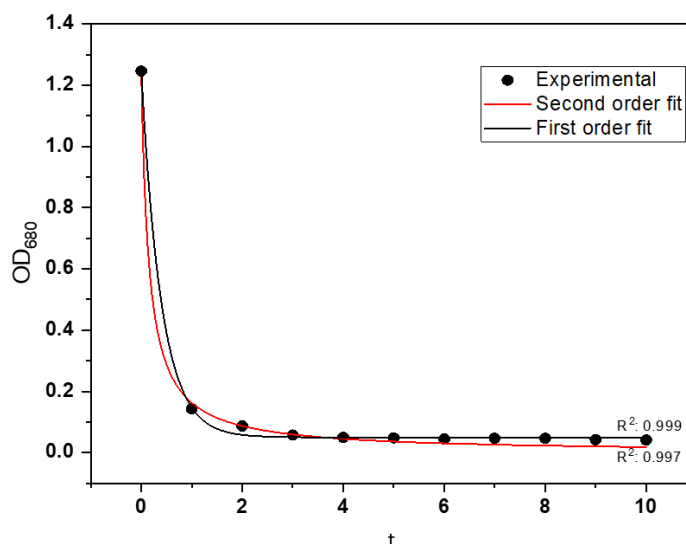


Figure 8 Decrease in the optical density (OD₆₈₀) of the microalgae suspension over time at the optimum dose of chitosan. The experimental data were fitted using the first- and second-order kinetic models

Pomegranate seed waste also achieved high harvesting efficiency, reaching 98.5% at 0.25 g L⁻¹ and pH 11. This finding demonstrates the potential of minimally processed plant waste as a flocculating material. Shrimp shell-derived chitosan was effective at a lower mass concentration and under mildly acidic conditions. However, this contrast should not be regarded as evidence of superiority because the two studies used different microalgal species, biomass concentrations, and harvesting protocols. Harvesting efficiency of the synthesized chitosan was comparable to that reported for several natural and bio-based coagulants while operating at a relatively low volumetric dosage. The coagulant was produced from shrimp shell waste, thereby combining microalgae harvesting with waste valorization. Nevertheless, a reliable comparison of technical performance would require the coagulants to be evaluated under identical biomass concentrations, water matrices, mixing conditions, and separation times. Therefore, this comparison demonstrates that shrimp shell-derived chitosan is a viable natural coagulant for harvesting *Chlorella vulgaris* without establishing a universal performance advantage over other materials.

4. Conclusions

This study shows that waste-derived chitosan from *Litopenaeus vannamei* shells can function as an efficient and structurally nondestructive biocoagulant for harvesting microalgae. The extracted chitosan exhibited moderate crystallinity (73.76%) and a relatively high degree of deacetylation (78.97%), providing sufficient active amine sites to promote electrostatic interaction with negatively charged *Chlorella vulgaris* cells. Under optimized operating conditions (10 mg L⁻¹ chitosan, pH 6, and 10 min contact time), harvesting efficiencies greater than 95% were achieved within a short separation time. Kinetic analysis revealed strong conformity to the first-order model, indicating that the OD₆₈₀ decay profile is well described by an exponential function under the tested conditions. The FTIR analysis of the harvested biomass confirmed the

preservation of the main functional group profile relative to the control, indicating that chitosan-assisted coagulation proceeds via adsorption-driven interactions without detectable alteration of the intrinsic chemical fingerprint of the cells. Structural integrity preservation is advantageous for downstream valorization in biofuel, protein, or broader biorefinery applications. Collectively, these findings position shrimp shell-derived chitosan as a promising and more sustainable alternative to conventional chemical coagulants, while also supporting the integration of microalgae harvesting into circular bioeconomy pathways. The mechanistic and kinetic insights obtained herein provide a basis for future scale-up and process optimization of bio-based coagulation strategies for microalgal bioprocessing.

Acknowledgements

This research was financially supported by The Faculty of Engineering, Diponegoro University, Indonesia through Flagship Research Grant 2026 under Grant Number 032/ Unggulan/Teknik Kimia/3/UN7.F3/PP/III/2026.

Author Contributions

H. Hadiyanto: Conceptualization, Supervision, Funding acquisition, Project administration, Writing – review & editing. **Indro Sumantri:** Methodology, Formal analysis, Investigation, Data curation. **Moch Djaeni:** Methodology, Validation, Writing – review & editing. **Nyoman Widiassa:** Supervision, Resources, Writing – review & editing. **S. Suherman:** Supervision, Resources, Writing – review & editing. **Zainul Akmar bin Zakaria:** Methodology, Validation. **Wahyu Diski Pratama:** Investigation, Data curation, Visualization. **Dzakwan Hafizh:** Investigation, Data curation. **Risyad Prasetya Muzaki:** Investigation, Data curation. **Wahyu Zuli Pratiwi:** Methodology, Formal analysis, Writing – original draft, Project administration.

Conflict of Interest

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

Supplementary Materials

Supplementary figures and tables are available online.

Declaration of AI

During the preparation of this work, ChatGPT 5.1 was used to improve readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

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