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Comparative Study on Flocculation Efficiency of Shrimp and Crab Chitosan for Irrigation-Ready Sludge

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Abstract: With the growing global threat of water scarcity, wastewater treatment and recycling have become essential to sustainable water management strategies across the world. The necessity of recycling water resources plays a crucial role in ecological planning and urban design, helping to mitigate the environmental and health risks associated with untreated wastewater. The current study explores the extraction and application of chitosan from shrimp and crab shells for the treatment of activated sludge, aiming to enhance its physico-chemical properties for sustainable use in irrigation. Seafood processing industries generate substantial waste, predominantly shells, which are often discarded, contributing to environmental pollution. This study capitalizes on these waste materials by extracting chitosan, a biopolymer with versatile applications, from shrimp and crab shells. The extraction involved demineralization, deproteinization, and deacetylation processes, followed by characterizing the resulting chitosan for yield, moisture content, solubility, water-binding capacity, fat-binding capacity, degree of deacetylation, and molecular weight. The extracted chitosan was then applied to activated sludge, with key parameters such as pH, total dissolved solids, total suspended solids, chemical oxygen demand, biochemical oxygen demand, nitrate, nitrite, ammonia, dissolved phosphorus, sludge volume index, electrical conductivity, and chloride content being measured. The results demonstrated that both shrimp and crab chitosan significantly improved the sludge's characteristics, with shrimp chitosan showing slightly better efficiency. The enhanced sludge exhibited properties suitable for agricultural applications, suggesting that chitosan could be a valuable tool for waste management and sustainable agriculture. This study highlights the potential of seafood waste as a resource, offering an eco-friendly solution to environmental pollution while contributing to the circular economy.

Keywords: Agriculture, Biopolymers, Crustacean waste, Environmental sustainability, Wastewater treatment, Chitosan

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Introduction

Water scarcity has become a pressing global concern, prompting nations worldwide to prioritize wastewater treatment and recycling as essential components of sustainable water management strategies. The imperative to recycle water resources underscores its significance in ecological planning and urban design, particularly in mitigating environmental and health risks linked to untreated sewage (Malisa *et al*., 2019). Municipal wastewater, comprising a complex mix of organic and inorganic substances, poses substantial environmental and health hazards if not properly treated (Singh *et al*., 2022). The presence of suspended solids, dissolved pollutants, pathogens, and nutrients necessitates effective treatment to prevent contamination of natural water bodies and soil.

India faces profound challenges in sewage management, with a significant portion of urban wastewater being discharged untreated into natural water bodies. This practice not only contaminates surface water but also threatens groundwater quality, exacerbating health risks and environmental degradation in densely populated urban areas (Akhtar *et al*., 2021). The escalation of industrialization and urban growth has led to a significant increase in activated sludge production. This sludge, characterized by its high-water content exceeding 95%, presents formidable challenges for storage and disposal. Effective dewatering and volume reduction of sludge are imperative due to its sheer volume and the limitations of conventional treatment methods. Activated sludge consists of negatively charged colloidal particles that remain stably dispersed in water, making their separation via traditional precipitation methods difficult (Saravanan *et al*., 2021). Efficient sludge conditioning and dewatering technologies are crucial not only for reducing water content and volume but also for minimizing the costs associated with sludge handling and disposal.

Among the various technologies employed, mechanical flocculation and conditioning have emerged as cost-effective solutions for separating sludge from water. However, the widespread use of traditional inorganic and synthetic organic flocculants raises concerns about their environmental impact and economic sustainability. In response, there has been growing interest in natural modified polymer flocculants, which offer environmentally friendly alternatives (Chowdhary *et al*., 2022).

Chitosan, derived from the shells of crustaceans such as shrimp and crab, has attracted considerable attention for its biodegradable, non-toxic, and eco-friendly properties. As the second most abundant natural polymer after cellulose, chitosan possesses active amino and hydroxyl groups. This feature enables the synthesis of high molecular weight polymer flocculants that significantly enhance flocculation performance in wastewater treatment and sludge dewatering processes (Hyrycz *et al*., 2022)

The increasing prominence of activated sludge production and the environmental challenges associated with its treatment underscore the urgent need for sustainable solutions. Chitosan has emerged as a front-runner among modern treatment solutions due to its versatility in addressing wastewater challenges. Particularly noteworthy is its effectiveness in binding contaminants and pathogens, thereby enhancing the efficiency of wastewater treatment processes. Shrimp and crab-derived chitosan, with its superior binding capacity owing to its high molecular weight and degree of deacetylation, has been extensively studied for its efficacy in purifying water (Chowdhry *et al*., 2022).

Chitosan's applications extend across diverse industries, including agriculture, pharmaceuticals, and environmental remediation, underscoring its multifaceted utility. Its antimicrobial properties are particularly advantageous in water purification, where it aids in the removal of contaminants and pathogens (Hameed *et al*., 2022). Addressing the challenges posed by wastewater treatment is not only essential for sustainable development but also for safeguarding public health globally. Integrating shrimp and crab-derived chitosan into wastewater treatment processes offers a promising avenue to enhance water quality and mitigate the environmental impacts associated with untreated sewage.

This study aimed to explore the differential efficacy of shrimp and crab-derived chitosan in wastewater treatment, emphasizing its pivotal role in advancing sustainable solutions amidst escalating global water challenges. By elucidating the unique properties and applications of these natural polymers, this research seeks to contribute to the development of effective and environmentally friendly solutions for wastewater management.

Materials and Methods

Collection and processing of sample:

Shrimp and crab waste was collected from vendor who has freshly sold the sea food in Vanagaram, Chennai, Tamil Nadu, India. The shells were then separated from other debris attached along with the waste. The shells were rinsed using warm distilled water and dried in an oven at 60℃ for 12 h for shrimp shells and 36 h for crab shells. The dried shells were broken to pieces for better surface reaction for various reaction solvents. It is then refrigerated at 4℃ until further use.

Shrimp chitin extraction:

The biopolymer was extracted from shrimp shells with slight modifications by the method of Ogretmen *et al.* (2022). The shells were demineralized with 5% hydrochloric acid in a 1:10 (w/v) ratio for 24 h and deproteinized with similar w/v ratio in 7% sodium hydroxide for 5 hours. After each step, the residue was pH neutralized. After neutralization, the chitin is oven-dried and stored in a cool, dry place for deacetylation process.

Crab chitin extraction:

The extraction process in fractionated crab shells were carried out with slight modifications by method of Hao (2021). The shells were deproteinized using 4% sodium hydroxide at 1:10 (w/v) for 2 h at 80℃. Followed by demineralization using 15% hydrochloric acid 1:15(w/v) for 3 h. Similar to shrimp chitin, the residue was neutralized at each step and oven-dried to get a moisture free chitin, which was stored until used.

Deacetylation of chitin:

The acetyl groups of shrimp and crab chitin was removed according to method of Ewais (2023) with modifications. Both chitins were treated separately with 60% NaOH at 110℃ for 5 h in 1:50 (w/v). The content was carefully filtered to avoid missing even the tiniest of flakes which may affect the overall yield. The resulting white substance was neutralized to pH 7 with distilled water and oven dried to remove moisture content and stored in a cool dry place for further use.

Characterization of chitosan:

Determination of yield: The yield of both shrimp and crab chitosan was estimated (Triunfo *et al.*, 2022) using the formula:

$$\text{Chitosan yield}(\%) = \frac{\text{dry weight of chitosan (g)}}{\text{dry weight of shell (g)}} \times 100$$

Determination of moisture content: Moisture content was determined according to the Association of Official Analytical Chemists (AOAC) standard method no. 930.15 (International, 2023).

Determination of solubility: 0.1g of chitosan was dissolved in 10 ml of 1% acetic acid and left for 30 min. It is then centrifuged for 10 min at 10,000 rpm. The supernatant was discarded and the undissolved pellet was dried at 60℃ for 24 h. Finally, the amount of residue was measured and the percentage of solubility was calculated (Ogretmen *et al.*, 2022).

Water binding capacity (WBC): 0.5 g of the sample and 10 ml of water was added in a centrifuge tube. It was vortexed for 1 min and incubated at room temperature for 30 min. Later, the tube was centrifuged at 3500 rpm for 25 min. The supernatant was descanted and weighed (Kadak *et al.*, 2023).

$$WBC(\%) = \frac{\text{Water bound}}{\text{Sample weight}} \times 100$$

Fat binding capacity (FBC): 5 different oils were used for this assay – soybean, canola, corn, sunflower, and olive (Adekanmi *et al*., 2020). 10 ml of respective oils were added with 0.5 g of sample. It is then vortexed for 1 min for proper dispersion. The contents were then left undisturbed at room temperature for 30 min. Centrifugation was performed at 3500 rpm for 25 min. The supernatant was discarded and the remaining pellet was weighed and FBC was calculated by using the following formula:

$$FBC(\%) = \frac{\text{Fat bound (g)}}{\text{Sample weight (g)}} \times 100$$

Degree of deacetylation (DDA): To determine the degree of deacetylation of chitosan, first derivative UV-spectrophotometer procedure was employed according to method of AI-Jbour *et al.* (2021), which is considered as the second most cost-effective way to calculate the degree of deacetylation followed by acid-base titration technique. A standard calibration curve at a maximum wavelength of 202 nm (λ_{max}) was plotted for N-acetylglucosamine at various concentrations. The chitosan solution was prepared at a concentration of 50 µg/ml. With the same wavelength, by applying square linear regression, the slope of the curve was obtained. Then the degree of deacetylation was obtained by applying the following equation:

$$\%DDA = \frac{[100 \times M1(C1 - C2)]}{[(M1 \times C1) - (M1 - M3) \times C2]}$$

Where; C1 and C2 are the concentration (µg/ml) of chitosan, and the concentration of N-acetylglucosamine (µg/ml) in the test solution, respectively, M1 is the relative molecular mass of N-acetylglucosamine unit (203.2 g/mol), and M3 is the relative molecular mass of chitosan sample as calculated from the pH of the solution, using a pKa of glucosamine equal to 6.8 and by applying Equations:

$$M3 = (f \times M2) + (1 - f) \times (M2 + 36.3$$

$$f = \frac{p}{1 + p}$$

$$p = 10^{pH - pKa}$$

Where; M2 is the relative molecular weight of glucosamine (197.62 g/mol), f is the fraction of ionized species in the aqueous phase, and p is the partition-coefficient that refers to the concentration ratio of un-ionized species.

Molecular weight: The intrinsic viscosity was calculated using Ubbelohde intrinsic viscometer. The equipment was maintained at temperature of 25°C using a water bath. All the solutions were filtered before use by using 0.45 µm pore size filter paper. The flow time of solvents, followed by chitosan solution was observed and measured. The experiment was conducted five times to ensure the accuracy and rinsed each time while changing solutions (Czechowska-Biskup *et al*., 2018). The Mark-Houwink equation determines the relationship between the intrinsic viscosity and average molecular weight of the polymer.

$$\eta = k.(M_w)^a$$

Where η – intrinsic viscosity, M_w- average molecular weight, K = 0.00181 cm3 /g and a = 0.93 at 25°C (Lewandowska, 2020).

Intrinsic viscosity can be calculated using following formula:

$$\eta_2 = \frac{\rho_2 t_2}{\rho_1 t_1} \times \eta_1$$

Where η_1- Viscosity of water, η_2- Viscosity of test sample, ρ_1- Density of water, ρ_2- Density of test sample, t_1 – Mean time of flow water, t_1 – Mean time of flow of test sample.

Collection of sewage sample and chitosan treatment:

The activated sludge was collected from sewage treatment plant, Chennai, Tamil Nadu, India. The sample was collected from the aeration tank in a sterile container and transferred to the lab immediately for further analysis. The sludge was mixed thoroughly and equally divided into three for shrimp chitosan treatment, crab chitosan treatment and control. Various physico-chemical parameters were considered for the present study. These are:

pH: To determine the pH, the samples and the control were taken in a clean glass beaker and tested using electrode of pH meter.

Total dissolved solids (TDS): 90 ml of evaporating dish capacity was cleaned, and 10 ml of filtered wastewater sample and control were run through Whatman number 1 filter paper. Using a hot water bath allowed the sample to evaporate completely. A desiccator was used to cool the evaporating dish. The total dissolved solids were calculated using standard formula:

$$TDS\left(\frac{g}{L}\right) = \frac{F2 - F1}{\text{Volume of sample}} \times 100$$

Where, F2 – Final weight of evaporating dish (g), F1 – Initial weight of evaporating dish (g).

Total suspended solids (TSS): Measured sewage sample volume was pipetted onto the seated glass fiber filter while stirring. The sample was pipetted from a point that was roughly halfway between the wall and the vortex and mid-depth in the container. The material that remains on a regular glass fiber filter following the filtration of a thoroughly blended sample is known as total non-filterable residue. The filter paper was weighed after cooling in a desiccator and drying in an oven at 103 to 105°C for at least an hour.

$$\text{Total suspended solids}\left(\frac{mg}{L}\right) = \frac{A - B}{\text{Sample Volume}} \times 1000$$

Where, A = weight of filter residue, B = weight of filter.

Chemical oxygen demand (COD): A 20 ml sample was placed in the reflux unit's flask. 10ml of potassium dichromate solution, a pinch of silver and mercuric sulphate, and 30 ml of sulphuric acid were added to the sample. The sample was made in duplicate, along with one blank setup (double distilled water). The Liebig condenser was attached to the flask's mouth, and the flask was heated on the heating mantle for 2 h to allow the contents to reflux.The sample flask was removed from the reflux unit and cooled. The sample contents were diluted to 150 ml by adding double distilled water. 2-3 drops of ferroin indicator solution were added to the sample flask and titrated with ferrous ammonium sulphate solution. When the end point was achieved, the contents of the sample flask changed colour from reddish to blue. COD of the sample was calculated using formula:

$$COD\left(\frac{mg}{L}\right) = \frac{(B - A) \times N \times 8}{V} \times 1000$$

Where, A= Volume of titrant used against sample; B= Volume of titrant used against blank; N= Normality of titrant (0.25); V = Volume of sample (ml).

Biochemical oxygen demand (BOD): Distilled water was diluted with 1 ml each of phosphate buffer, magnesium sulphate, calcium chloride, and ferric chloride solutions, and the mixture was allowed to aerate for 30 min. The pH was corrected to 7.0 using 1N sulfuric acid and then diluted with BOD-free distilled water. The sample was poured into two sets of 300 ml BOD bottles. 2 ml of manganous sulphate, 2 ml of alkaline potassium iodide, and 1 ml of allylthiourea were combined, agitated, and precipitate was seen. 2 ml of Conc. sulfuric acid was used to dissolve the precipitate. One set was tested immediately for dissolved oxygen, while the other was incubated at 20°C for 5 days. After incubation, the sample was transferred to a conical flask, 2 drops of starch indicator were added, and titration against sodium thiosulphate was done until the solution changed from blue to colourless.

$$\text{Dissolved oxygen}\left(\frac{mg}{l}\right) = \frac{V1 \times N \times 8}{V2 - V3} \times 1000$$

Where, V1 = Volume of titrant (ml); N = Normality of titrant (0.025N);V2 = Volume of sampling bottle after placing the stopper (ml); V3 = Volume of manganous sulphate solution + alkaline potassium iodide solutions added (ml).

Similarly, the second set of BOD bottle which was incubated at 20°C for 5 days in BOD incubator was also analysed and value was noted. BOD was calculated using the formula:

$$BOD5 = (D_0 - D_5) \times \text{Dilution factor}$$

Where, D_0 = Initial dissolved oxygen in the sample (mg/l); D_5 = Dissolved oxygen left out in the sample after 5 days incubation (mg/l).

Nitrate: Standard nitrate solutions at various concentrations were prepared. 2 ml of standard/sample/blank were taken and 1 drop of sulphite-urea reagent was added. While placing the tubes in a cold environment, 2 ml of antimony reagent was added and swirled firmly. After 4 min, chromotrophic acid reagent was added followed by concentrated sulphuric acid to make up to 10 ml. It was incubated at room temperature for 45 min. The tubes were gently mixed avoiding gas bubbles. Absorbance was measured at 410 nm in a spectrophotometer and a graph was plotted. The concentration of nitrate was calculated using the formula:

$$NO_3\left(\frac{mg}{L}\right) = \frac{NO_3\,\mu g \text{ in } 10 \text{ ml}}{\text{sample volume}}$$

Nitrite: pH neutralized samples were taken and diluted to 50ml. Different nitrite concentrated solutions were prepared and kept as standard. 1ml of sulphanilamide reagent was made to react with sample and standard for 3 min. Followed by this, 1 ml of N – (1 naphthyl) – ethylene diamine - di hydrochloride solution was added to all the reaction flask. The concentration of nitrite was calculated using the formula:

$$NO_2\left(\frac{mg}{L}\right) = \frac{NO_2\,\mu g \text{ in } 52 \text{ ml}}{\text{sample volume}}$$

Ammonia: Sample preparation involved adding 500 ml of sample to a distillation flask, followed by 25 ml of borate buffer solution. The pH was adjusted to 9.5 with 6 N sodium hydroxide. Distillation occurred at 6 ml/min with the delivery tube submerged 2 cm below the surface of the acid receiving solution. A 300 ml distillate was collected into a 500 ml dried conical flask containing 50 ml of indicating boric acid solution. The distillate was then titrated against 0.02 N sulphuric acid until a pale lavender colour indicated the end point. The burette reading noted the amount of titrant used for determining ammonia nitrogen, which was then computed using the appropriate equation. A blank was carried through all steps of the procedure.

$$\text{Ammonia}\left(\frac{mg}{L}\right) = \frac{(A - B) \times 200}{\text{sample volume}}$$

Where, A=Volume of H_2SO_4 titrated for sample(ml), B= Volume of H_2SO_4 titrated for blank(ml).

Dissolved phosphorous: To 100 ml of samples, 1 drop of phenolphthalein indicator was added followed by a strong acid reagent till pink colour disappears. The solution was boiled for 90 min and neutralized using 3 N NaOH. Standard phosphorous solution was prepared by diluting various concentration of phosphorous. 2 ml of ammonium molybdate solution and 0.5 ml of stannous chloride was added step by step to this and each solution is diluted to 100 ml and left undisturbed for 10 min. A calibration curve was plotted using the standards at a wavelength of 690 nm using a spectrophotometer. The concentration of phosphorous was calculated with the formula:

$$P\left(\frac{mg}{L}\right) = \frac{P(mg) \times 1000}{\text{Sample in ml}}$$

Sludge volume index (SVI): 1 litre sample was deposited in a settling column under stirring, and the suspension was allowed to settle. The suspension temperature was kept constant throughout the test as it was in the basin from which the sample was obtained. The suspension occupied by volume was measured at a 30 min time interval. SVI was calculated using the formula:

$$SVI = \frac{\text{Settled sludge volume}}{\text{Non} - \text{filterable residue}} \times 1000$$

Electrical conductivity: Electric conductivity can be measured by electric conductivity meter. The samples and control were tested using electrode of electric conductivity meter.

Chlorides: 10 ml sample was placed in a clean conical flask and 5-6 drops of potassium chromate indicator was added to the sample and titrated against 0.02 N silver nitrate solution. The titration was stopped when a persistent brick red colour end point was reached in the sample. Burette reading was noted for the amount of titrant consumed in determination of chloride and computed in the equation for calculation using formula:

$$\text{Chloride}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{V \times N \times 35.457}{S} \times 1000$$

Where, V = Volume of titrant (ml), N = Normality of titrant, S = Volume of sample (ml).

Results and Discussion

The comparative analysis revealed that both shrimp and crab chitosan significantly improved the physico-chemical properties of treated activated sludge. Globally every year 8 million tons of wastes are generated by sea-food processing. These wastes are either dumped back into ocean or into landfill which has effects on soil, water and air. Instead, these waste are a source of value-added product which can be implicated in various field of industries (Topic Popovic *et al*., 2023). In the present study, one such value-added by product was extracted from shrimp and crab shells. Chitosan is a multipurpose polymer well known and used around the world for their environment friendly properties. During the extraction of chitin, the shells of crab are demineralized with higher amount of HCl when compared with shrimp shells, as they contain higher amount of minerals, especially $CaCO_3$ in them (Endang *et al*., 2019) which becomes a crucial step for their extended drying period of shells before process also. In contrast, the shrimp shells contain higher amount of protein when compared with crab shells which was a main concern during deproteinization (Isa *et al*., 2012).

Though the differences were less significant, yield of shrimp chitosan showed better result than crab chitosan (Table 1). Shrimp shells contain high amount of chitin which gradually paves the way for higher yield of chitosan. Pakizeh *et al.* (2021) elaborates on how commercial chitosan are mostly extracted from shrimp. The yield of shrimp in the study matches with reports of Ogretmen *et al*. (2022). Crab shells yielded a good amount of chitosan which is similar to findings of Hao *et al*. (2021).

During storage, variations in the material's moisture content can affect the physic-chemical and mechanical properties of chitosan (Sreelekshmi *et al*., 2022). The moisture of extracted shrimp chitosan is within the standard range <10 and it significantly less than that reported by Anggreini *et al*. (2023). They have further mentioned that the antimicrobial property of chitosan is mainly affected by the water content in them. No vast difference was found between the moisture content of shrimp and crab chitosan (Table 1). Similar crab chitosan moisture was obtained by Pratama *et al*. (2023). Solubility is an important aspect of chitosan for their enhanced properties and applications (Harugade *et al*., 2023). Chitin is most importantly deacetylated to chitosan in order to improve its solubility, so as to implement in various research studies. Physical, chemical and biological properties mainly depend on the solubility of product. In the present investigation, the solubility of both crustacean chitosan is notable (Fig. 1). The results almost matched with Parthiban *et al*. (2017).

The water binding capacity and fat binding capacity of chitosan is mainly influenced by the sequence of demineralization, deproteinization and deacetylation (AI-Hassan *et al*., 2016). Graphical representation of water and fat binding capacity is given in Figure 2. The water binding capacity of shrimp and crab chitosan in the present study is lower than that reported by Sultana *et al*. (2020). The fat binding capacity of shrimp chitosan in the study matches with the findings of Apetroaei *et al*. (2019). From the results, we can see that shrimp chitosan have actively absorbed the water and oil more effectively than crab chitosan. This might be due to the concentration variations of solvents due to the demineralization and deproteinization for shrimp and crab chitin. While analysing the fat binding capacity, it was observed that oils containing unsaturated fatty acids have been more attracted

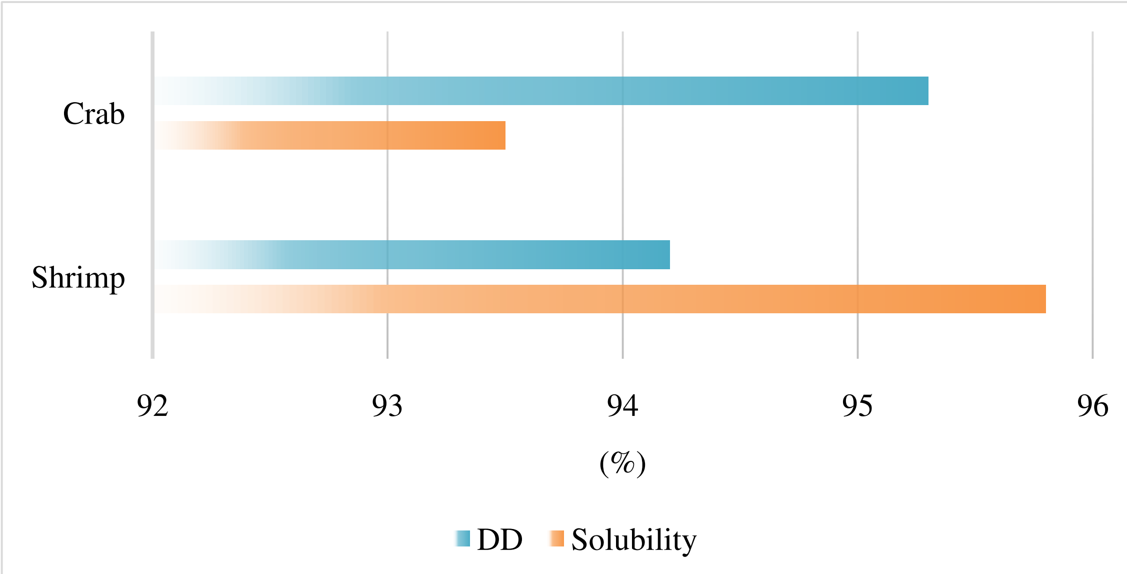

Fig. 1: Degree of deacetylation and Solubility of shrimp and crab chitosan.

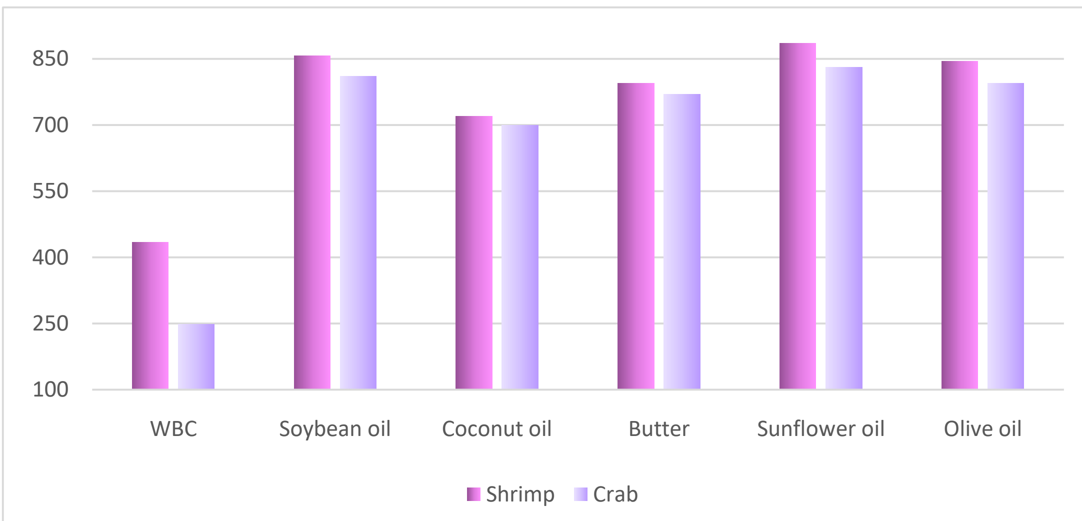

Fig. 2: Water and Fat binding capacity of extracted shrimp and crab.

than saturated fatty acids. Saturated fatty acids are compact with straight chains which gives less space for chitosan to fill in through, while unsaturated fatty acids are bent due to the presence of cis-arranged single or double bonds (Silva *et al.*, 2021).

The UV-Vis spectroscopic method is one of the widely utilized method in order to evaluate the degree of deacetylation of chitosan (Sanchez-Machado *et al.*, 2024). They are known to provide results with high sensitivity, accuracy and precision (Jiang *et al.*, 2017). Figure 1 shows the degree of deacetylation of both shrimp and crab chitosan. The measured degree of deacetylation values in this study varies between the sources. Though the variation is only minimal, the properties might have greater effect over them. The effect of degree of deacetylation was further discussed by (Bhardwaj *et al.*, 2020), that properties such as chelating and flocculation ability, tensile strength, ion-exchange capacity, immune activity, and especially solubility and viscosity of the polymer has the greater impact with respect to degree of deacetylation. From the results, it was found that shrimp chitosan has higher degree of deacetylation than crab chitosan. With same deacetylation procedure, this difference is majorly might be due to crystalline nature of their respective chitin. Crab chitin is found to be denser crystalline in nature when compared to shrimp chitosan, whose crystallinity index is found to be more accessible (Lupo, 2019) which makes it to deacetylate in much faster pace.

Molecular weight of chitosan is an important aspect along with the degree of deacetylation of the biopolymer. It is greatly influenced by the raw material composition and the process of conversion from chitin to chitosan. The intrinsic viscosity of the compound represents the molecular weight of the same. Haemostatic, bacteriostatic and fungistatic property of the compound is dependable on the degree of viscosity (Ardean *et al*., 2021). In this study, shrimp chitosan was found to have higher molecular weight when compared to crab chitosan (Table 1). It was confirmed that this was the only parameter that had vast difference between the two source of chitosan. (Narudin *et al*., 2020) cited the results which were less than the present study. Like degree of deacetylation, the crystallinity of the substrate plays a major role in viscosity, which in turn towards the molecular weight.

Table 1: Properties of chitosan

Source	Yield (%)	Moisture (%)	Molecular weight (KDa)
Shrimp	17.6 ± 0.3	8.89 ± 0.02	18.15 ± 2.3
Crab	12.7 ± 0.4	8.77 ± 0.03	6.53 ± 1.7

This study aimed to evaluate the effectiveness of shrimp and crab chitosan in treating activated sludge and assess the suitability of the treated sludge for irrigation purposes. Several key parameters were analysed and illustrated in Table 2. The results indicate that sludge treated with shrimp chitosan is superior and meets the criteria for safe irrigation use.

The pH remained consistent at 7.06 across the control, shrimp chitosan, and crab chitosan treatments, which is ideal for irrigation. Maintaining a neutral pH in treated sludge is crucial as extreme pH levels can affect nutrient solubility, soil microbial activity, and overall plant health. Neutral pH ensures that essential nutrients like nitrogen, phosphorus, and potassium remain available to plants while avoiding issues like soil acidification or alkalization. This stability in pH with chitosan treatment is consistent with findings from other studies that emphasize the ability of chitosan to maintain or slightly buffer pH levels in various water treatment applications (Rinaudo, 2006; Badawy *et al*., 2011). The consistent pH levels suggest that chitosan treatment does not introduce significant acidity or alkalinity, making the treated sludge suitable for various crop types that prefer a neutral growing medium.

The significant reduction in Total Dissolved Solids (TDS) from 719 mg/l in the control to 607 mg/l with shrimp chitosan treatment demonstrates the efficacy of shrimp chitosan in lowering dissolved salts and minerals in the sludge. High TDS levels in irrigation water can lead to soil salinization, which adversely affects plant growth, soil structure, and water absorption (Kumar *et al*., 2021). The effectiveness of shrimp chitosan in reducing TDS is likely due to its ability to adsorb and remove a wide range of dissolved ions and organic molecules, as documented in studies where chitosan was used for water treatment (Rinaudo, 2006; Alishahi and Aider, 2012). The reduction in TDS by shrimp chitosan-treated sludge makes it safer for irrigation, reducing the risk of salt buildup in the soil, which is critical for the sustainable use of treated water in agriculture.

Shrimp chitosan reduced Total Suspended Solids (TSS) more effectively than crab chitosan,

Table 2: Physico-chemical analysis of activated sludge treatment

Parameters	Unit	Control	Shrimp chitosan	Crab chitosan
pH	@ 25℃	7.06	7.06	7.10
Total dissolved solids	mg/l	719	607	615
Total suspended solids	mg/l	125	65	75
Chemical oxygen demand	mg/l	1200	800	950
Biological oxygen demand	mg/l	202	179	185
Nitrate	mg/l	17	19	18
Nitrite	mg/l	0.9	1	1.1
Ammonia	mg/l	137	238	220
Dissolved phosphorous	mg/l	5	58	60
Sludge volume index	-	63.6	85.6	80.3
Electrical conductivity	µmhos/cm	1357	1289	1305
Chlorides	mg/l	319.1	290.5	300

indicating its superior flocculation capabilities. High TSS in irrigation water can clog irrigation systems, reduce soil permeability, and impede plant root oxygenation (Stavi *et al*., 2021). The higher efficiency of shrimp chitosan in reducing TSS can be attributed to its molecular structure, which allows for better aggregation and sedimentation of suspended particles (Amal *et al*., 2023). This reduction in TSS is significant for irrigation purposes, as it ensures that the treated sludge has lower turbidity and fewer particulates, making it more suitable for use in drip and sprinkler irrigation systems, which are sensitive to clogging.

The reduction in Chemical Oxygen Demand (COD) from 1200 mg/l in the control to 800 mg/L with shrimp chitosan highlights its effectiveness in decreasing the organic load in the treated sludge. COD is a critical parameter for assessing the organic pollution level in wastewater, and high COD levels can lead to oxygen depletion in soil and water bodies, adversely affecting plant and aquatic life (Patel and Bhatt, 2022). The ability of shrimp chitosan to reduce COD is consistent with its well-documented adsorption properties, which enable it to bind with and remove organic pollutants (Kurniawan *et al*., 2024). The substantial reduction in COD with shrimp chitosan treatment indicates that the treated sludge is less likely to contribute to soil and water pollution, making it more suitable for reuse in agriculture.

The decrease in Biological Oxygen Demand (BOD) from 202 mg/l in the control to 179 mg/l with shrimp chitosan suggests improved biodegradability of the organic matter in the treated sludge. BOD measures the amount of oxygen required for microbial degradation of organic matter; lower BOD indicates a lower oxygen demand, which is beneficial for maintaining soil and water quality (Lokman *et al*., 2021). The reduction in BOD with shrimp chitosan treatment aligns with other studies that have shown chitosan's effectiveness in enhancing the biodegradability of organic pollutants by facilitating their aggregation and subsequent removal. This makes shrimp chitosan-treated sludge more suitable for irrigation, as it reduces the risk of oxygen depletion in the soil, which can harm plant roots and beneficial soil microorganisms.

The slight increase in nitrate levels from 17 mg/l in the control to 19 mg/l with shrimp chitosan could be beneficial for plant growth, as nitrate is a readily available form of nitrogen that is crucial for plant nutrition. Nitrate is essential for photosynthesis and protein synthesis, and its availability in irrigation water can enhance crop yields (Li *et al*., 2009). However, it is important to manage nitrate levels carefully to prevent leaching into groundwater, which can cause environmental concerns like eutrophication (Wang and Li, 2019). The observed increase in nitrate suggests that shrimp chitosan-treated sludge could potentially enhance soil fertility, but it should be applied judiciously to prevent negative environmental impacts.

The increase in nitrite levels from 0.9 mg/l in the control to 1 mg/l with shrimp chitosan is within acceptable limits for irrigation water. Nitrite is an intermediate in the nitrogen cycle, and its presence indicates ongoing nitrification processes (Heil *et al*., 2016). While nitrite can be toxic to plants at high concentrations, the levels observed here are unlikely to cause harm and may eventually be converted to nitrate by soil bacteria, contributing to the nitrogen available for plant uptake (Hakeem *et al*., 2017). The slight increase in nitrite with shrimp chitosan treatment suggests that it does not significantly disrupt nitrogen cycling, maintaining the balance of nitrogen forms in the treated sludge.

The significant increase in ammonia levels from 137 mg/l in the control to 238 mg/l with shrimp chitosan warrants careful management. Ammonia is a valuable nitrogen source for plants but can become toxic at high concentrations and may volatilize, leading to air quality issues (Skorupka and Nosalewicz, 2021). The elevated ammonia levels could be managed by applying the treated sludge in conjunction with nitrification inhibitors or using it as a slow-release fertilizer to reduce the risk of ammonia volatilization and improve nitrogen use efficiency (Li *et al*., 2022). Despite the increase in ammonia, shrimp chitosan-treated sludge could still be beneficial for agriculture if applied under controlled conditions to avoid negative impacts on crop health and the environment.

The increase in dissolved phosphorus from 5 mg/l in the control to 58 mg/l with shrimp chitosan raises concerns about potential eutrophication if the treated sludge is not managed properly. However, phosphorus is also a key nutrient for plant growth, and its presence in irrigation water can enhance soil fertility and crop yields (Itelima *et al*., 2018). The challenge lies in balancing the benefits of increased phosphorus availability with the need to prevent its runoff into water bodies, where it can contribute to algal blooms and water quality degradation. The higher dissolved phosphorus levels with shrimp chitosan treatment suggest that it could serve as a valuable nutrient source for crops if applied at appropriate rates and monitored to prevent environmental impacts.

The improvement in Sludge Volume Index (SVI) from 63.6 in the control to 85.6 with shrimp chitosan indicates better settleability of the sludge. A lower SVI reflects better sludge compaction and reduced bulk, which is advantageous for sludge handling and application (Zaidi *et al*., 2022). Shrimp chitosan's ability to enhance sludge settleability can be attributed to its strong flocculation properties, which promote the aggregation of fine particles into larger flocs that settle more rapidly (Lichtfouse *et al*., 2019). This makes shrimp chitosan-treated sludge easier to manage and apply as a soil amendment, reducing the volume of sludge that needs to be handled and improving the efficiency of sludge management in agricultural settings.

The reduction in Electrical Conductivity (EC) from 1357 µmhos/cm in the control to 1289 µmhos/cm with shrimp chitosan indicates a decrease in the total ion concentration in the treated water. Lower EC values are crucial for preventing soil salinity, which can impair plant water uptake and reduce crop yields (Zorb *et al*., 2019). The slightly better performance of shrimp chitosan in reducing EC compared to crab chitosan suggests its greater efficacy in removing dissolved salts, making the treated sludge safer for irrigation, especially in salt-sensitive crops. This reduction in EC aligns with other studies that have demonstrated the ability of chitosan to reduce the ionic content of treated water.

The reduction in chloride levels from 319.1 mg/l in the control to 290.5 mg/l with shrimp chitosan treatment underscores its effectiveness in removing excess salts from the sludge. High chloride concentrations in irrigation water can lead to soil salinization, which can inhibit plant growth, cause leaf burn, and disrupt nutrient uptake (Syed *et al*., 2021). The superior performance of shrimp chitosan in reducing chloride levels suggests that it can produce treated water that is safer for long-term irrigation use, particularly in areas where soil salinity management is critical for maintaining crop productivity.

Conclusion

The comprehensive analysis of the treated sludge parameters demonstrates that shrimp chitosan is more effective than crab chitosan in improving the quality of activated sludge for irrigation purposes. The significant reductions in TDS, TSS, COD, BOD, EC, and chloride levels, coupled with the stable pH and enhanced sludge settleability, indicate that shrimp chitosan-treated sludge is not only safe but potentially beneficial for agricultural use. The increases in ammonia, nitrate, and dissolved phosphorus levels highlight the need for careful management when applying this treated sludge to prevent environmental impacts. Overall, shrimp chitosan shows great promise as a flocculant for producing irrigation-safe water from activated sludge, with superior performance across multiple key parameters, making it a viable option for sustainable agriculture.

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