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Utilization of Fungal-Derived Chitosan from *Flammulina Filiformis* (Enoki Mushroom) and *Pleurotus Ostreatus* (Oyster Mushroom) as a Natural Coagulant in Wastewater Treatment

Nur Afiqah Sahira Saiful[1], Nor Faizah Razali[1]*

[1] *Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia, 84600Muar, Johor, Malaysia*

*Corresponding Author: faizahr@uthm.edu.my
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Abstract

The objective of this study is to obtain a stable extracted chitosan material to ensure its application as a natural coagulant used to treat wastewater. The stability and potential coagulating capacity of the extracted chitosan were investigated through a series of analyses on its degree of deacetylation (DDA) using Fourier Transform Infrared Spectroscopy (FTIR), its charge potential through Zeta-potential measurement, and its flow potential through rheological analysis. Previous studies used Enoki mushroom as fungal-derived however, on characterisation methods, were conducted independently from each other. This study integrates all three characterisation methods to examine the interrelationship between each parameter to obtain stable chitosan material to ensure its potential as a strong coagulant and flocculating agent. This research works on the use of chitosan derived from fungi, specifically enoki mushrooms (*Flammulina filiformis)* and oyster mushrooms (*Pleurotus ostreatus*), as a natural coagulant in treating wastewater. The purpose of this research is to derive chitosan from enoki and oyster mushrooms. Then, its physicochemical property was determined and tested for its coagulation efficacy as an alternative to conventional alum. Chitosan was derived through various steps, including deproteinization, treatment with acid, decolorization, and deacetylation. The derived chitosan was analyzed using (FTIR), Zeta potential, rheology test, and yield test. Coagulation efficacy was determined using jar test experiments at differing pH levels, wherein turbidity levels served as indicators. The findings show that chitosan derived from enoki mushrooms had chitin yields of 6.50%, having much higher positive surface charges, with zeta potential measurements above +70 mV, portraying high electrostatic attraction forces. Zeta potential measurements presented much smaller values, merely +50 mV. Rheological tests revealed that all samples had high shear rates, reducing viscosity values ranging from 100 Pa.s to 10-1-10-3 Pa.s when increased. Jar test experiments revealed chitosan derived from fungi having high turbidity removal rates, about 90-96%, almost comparable to those derived using alums. In general, this research concludes that chitosan derived from enoki mushrooms possessed high

surface charges, superior coagulation rates, and is thus viable and promising as sustainable, eco-friendly natural coagulants.

1. Introduction

Wastewater is a used water that contained manmade substances such as food scraps, oils, soaps, and chemicals [1]. There are 3 types of wastewater which are domestic sewage, industrial sewage, and storm sewage. Wastewater treatment is a process of removing the impurities from the wastewater or sewage, before it being released to any water sources or reservoir such as rivers, lakes, estuaries and oceans [2]. Wastewater treatment has 3 levels of treatment and might be more based on the company treatment which are primary, secondary, and tertiary process. The primary treatment involving the sedimentation process which coagulation, neutralization and flocculation will remove a large contaminants in wastewater. Gravity and mechanical forces are crucial in this stage as they will making sure contaminants can be float, clump with each other and settled at the bottom of the tanks [3].

Next is the secondary stage where trickling filters with secondary settling tanks, activated sludge, and modifications with final settling tanks, intermittent sand filters, and stabilization ponds [4]. This stage preferred to called as Biological treatment of wastewater as it involves the using of microorganism such as bacteria to eliminate and degrade the organic compound that remained in the wastewater [5]. In this stage, the wastewater in the activated sludge tank will be left for several days in 3 conditions which are aerobic, anoxic and anerobic for let the impurities to be removed [6]. The bacteria that lived in the tank or reactor has been supplied with enough organic matter and nutrients for the impurities removal that will results of the reduction of COD and BOD reading in the wastewater [6][7].

Finally, the tertiary stage which is the final cleanup setup where in this process, the removal of bacteria, nutrients, or chemical will be done to prevent for them to bring harm to the environment [8]. This stage can be the extension of the previous stage treatment as it will further stabilize the oxygen-demanding material in the wastewater or to be exact nitrogen and phosphorus removal [4]. Hence, water quality can be determined by parameters which are from the physical and chemical properties of the quality of the water which are dissolve oxygen (DO), turbidity, coliforms, turbidity, pH levels, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) [2].

1.1 Coagulation

This study is focus on the primary and preliminary stage where the coagulation and flocculation take part. Coagulation is a critical process in the primary treatment phase of wastewater treatment, where chemicals known as coagulants are added to destabilize suspended solids and allow them to group and settle as flocs. It significantly reduces turbidity, organic content, and contaminants from water [9]. Traditional synthetic coagulants such as aluminum sulfate (alum) and polyacrylamides have been widely used due to their rapid response and effectiveness. But growing fears about their toxicity, costliness, low biodegradability, and potential to form toxic sludge have rendered them doubtful for long-term sustainability and safety [10]. Therefore, in this study, we aim to investigate the extraction and application of chitosan derived from enoki (*Flammulina velutipes*) and oyster (*Pleurotus ostreatus*) mushrooms as a natural coagulant and adsorbent for wastewater treatment. This research explores the potential of fungal-derived chitosan as a sustainable, eco-friendly alternative to conventional synthetic coagulants in addressing water pollution challenges.

1.2 Method study

This research used an experimental method to isolate chitosan from the Enoki mushroom species *Flammulina filiformis* and the Oyster mushroom species *Pleurotus ostreatus* using a number of chemical processing techniques, which include deproteinization, acid treatment, decolorization, and deacetylation. The isolated chitosan was then processed to produce coagulant solutions. The physicochemical characteristics of the extracted chitosan were also determined by analyzing the Fourier Transform Infrared Spectroscopy results, Zeta potential analysis, Rheological analysis, as well as yield analysis. Lastly, the efficiency of using mushroom chitosan as a bio-coagulant was also determined using a jar test experiment with real wastewater.

1.3 Chitosan

Chitosan has long been known for its high efficacy as a biological coagulant for water and wastewater treatment due to its biodegradability, non-toxicity, and high coagulation power. Nonetheless, most related studies on this substance were previously carried from chitosan obtained from crustacean biota like shrimp and crab shells [11][12]. Even so, while this kind of chitosan possesses high coagulation efficacy, its usage poses various drawbacks such as being allergenic in nature, prone to seasonal fluctuations in supply, and needing highly intensive chemical processing for extraction. Hence, other uses for chitosan such as those derived from fungi have

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become considered and yet insufficient studies and information have been produced on their use as wastewater treatment coagulants yet [13].

1.4 Coagulation performance of conventional chemical coagulants and bio-based coagulants.

A study that used *Cactus Opuntia* pads and *Moringa oleifera* as plant-extract was examined by using coagulant in raw water treatment that have shown that alum achieved the highest turbidity removal compared to *Moringa oleifera* with a reading of 3.2 NTU while *Cactus Opuntia* had a turbidity reading of 3.0 NTU [14]. However, all three turbidity readings reached the lower limit set by WHO guidelines of 5 NTU. This shown that plant-extract coagulant also able to be used to treat the wastewater that brings more benefits such as sustainability compared to alum that produced toxic sludge towards the treated water. Mousavi et al. (2022) has stated that there is a study that produced hybrid plant-extract + coagulant and resulting a highest turbidity removal to be compared to conventional coagulant which is Alum [15]. The research stated that by increasing the concentration of the coagulant, the plant-extract herbs species has proved that turbidity measurement was improved from 20% to 92%, which was a huge jump of positive results. Table 1 below shows the difference perspective of conventional coagulants which is chemical coagulant and previous studies bio-chitosan coagulant.

Table 1 The difference perspective of conventional coagulants (chemical-derived) and bio-chitosan coagulant.

No.	Perspective	Conventional Coagulants (alum, $FeCl_3$)	Bio-Chitosan Coagulant	References
1.	Mechanism	Charge neutralization and sweep flocculation by formation of $Al(OH)_3$ / $Fe(OH)_3$ percipitates enmeshing colloids	Charge neutralization and polymer bridging by interacting the positive charge amino group with negative charge particles	[16] [12]
2.	Sources	Non-renewable mineral sources	Renewable bio-based resources (fungi, biomass)	[17][18]
3.	Example	Aluminium Sulfate (alum), $Al_2(SO)_4$ Ferric Chloride, $FeCl_3$	Chitosan from fungal sources Fungi wall (mushrooms) Creustacean shells	[16][17]
4.	Performance	High turbidity of removal but pH dependent	Comparable efficiency at lower dosage	[17][19]
5.	Health concerns	Residual may pose health risk	Non-toxic, biocompatible	[16]
6.	Environmental impact	Produced large toxic sludge, disruption of water pH, high operational cost, and adverse environmental impact	Produced less sludge, less toxic, and more sustainable and eco-friendly solution to water treatment	[20]
7.	Cost	Low chemical cost yet high sludge treatment cost	High cost but works better at lower doses make it a cost-efficient and lower sludge management cost.	[17] [21]
8.	Limitations	pH sensitivity and residual metals	Extraction complexity	[6]

From this study, it shown that plant-extract able to work the same as alum but in sustainable and eco-friendly way.

2. Methodology

All the strategies, procedures and techniques used in conducting research and collecting data will be elaborated with previous study references for gaining the natural coagulant which are from enoki (*Flammulina filiformis*) and oyster (*Pleurotus ostreatus*) mushrooms. It is a detailed method on how gaining a chitosan from the process of recovery of chitin from both mushroom until obtaining the chitosan from the obtained chitin. This chapter also provide testing technique that will involve several types of equipment to identify the presence of the compound in the chitosan itself and the test of the chitosan performance in the real-life action which are Jar Test for coagulation and flocculation process. Figure 1 shows the flow chart of the production of chitosan mushroom-based.

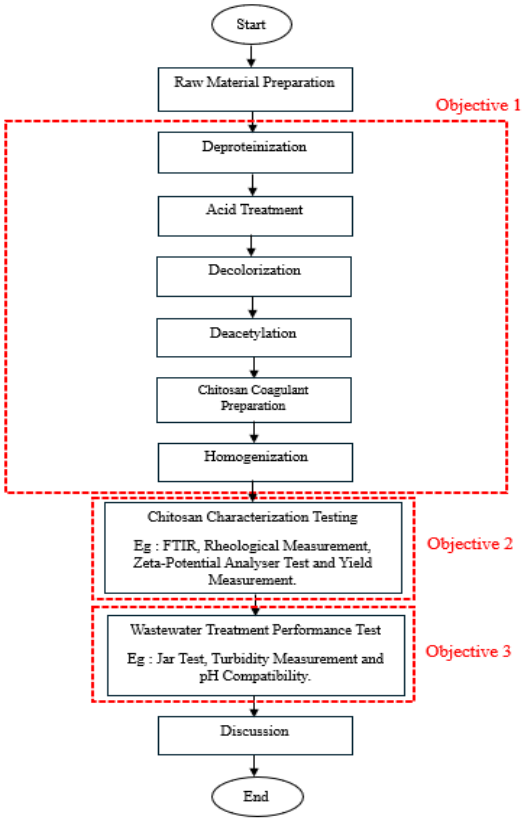

Fig. 1 *Flow chart of the production of chitosan mushroom-based.*

2.1 Procedure

Extraction of chitosan from enoki mushroom (*Flammulina filiformis)* and oyster mushroom (*Pleurotus ostreatus)* was done using a step-wise chemical process. First, the mushroom powder was treated with deproteinization involving soaking in a solution of 2M sodium hydroxide (NaOH) at a higher temperature and with continuous agitation. This resulted in the precipitation of proteins and soluble organic matter, forming an alkaline-insoluble residue. This residue was filtered and repeatedly washed using distilled water until neutral pH was reached. The residue was thereafter kept in a moist condition under chilling before it could be processed [22].

The alkaline-insoluble residues were further treated with dilute acetic acid solutions and heat to remove the remaining mineral residues and the acid-soluble fractions. The filtered samples were then thoroughly washed to achieve a neutral pH, resulting in chitin in a moist condition. The chitin was then purified through the process of decolorization using pure acetone, resulting in the removal of lipids, fats, oils, and hydrophobic pigments, which cause the material to appear dark. The samples were then filtered and stored as moist samples.

Subsequent to this, the chitin was subjected to a deacetylation process utilizing a concentrated solution of sodium hydroxide at elevated temperatures to produce chitosan [22]. The chitosan produced was filtered and oven-dried to obtain a powder of chitosan derived from fungi. For the production of chitosan coagulant solution, a predetermined quantity of chitosan powder was dispersed in 1% acetic acid to obtain a chitosan solution. The last process involved the homogenization of chitosan solution to ensure it is evenly dispersed and has equal properties, and it becomes a liquid chitosan coagulant.

2.2 Fourier Transform Infrared Spectroscopy (FTIR)

The dried chitosan were analyzed by using FTIR to confirm the characterization functional groups [22]. This is to ensure the functional group is tally with the synthetic one so that it will be functioning as the synthetic coagulant yet much safer to use it. This FTIR testing is especially for identify the Degree of Deacetylation (DDA) is calculated by using absorbance values in between 1600-1650 cm-1 and 3500-3200 cm-1 based on the formula :-

$$DDA\ \%=96.67-[26.486\times A_{(1600-1650)}/A_{(3200-3500)}\ ] \quad (1)$$

And

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$$DDA\ \%=100-[A\_1655/A\_3450 \times 100/1.33] \quad (2)$$

Where,
DDA (%) = Degree of Deacetylation
A1600-1650 = absorbance at Amide I band (C=O stretching vibration)
A3500-3200 = absorbance at O–H and N–H stretching vibration

Noted that, the relationship for FTIR testing is ; the higher the DDA (.70%), the better the solubility and adsorption power [22]. The method for this testing is the dry chitosan will be mixed with potassium bromide (KBr) in a 1:5 ratio. It then being pressed into a pellet and scan in FTIR.

2.3 Zeta-Potential Analyzer test

The zeta potential is then analyzed by using Zeta-potential analyser for the surface charge properties of chitosan derived from fungal sources of *Flammulina filiformis* (enoki mushroom) and *Pleurotus ostreatus* (oyster mushroom). Analysis of zeta potential is widely utilized as an analysis method for characterizing chitosan based on its electrokinetic properties in an aqueous environment [23][24]. Analysis is done using a Malvern zetasizer purchased through an ELS principle called electrophoretic light scattering, which is widely used for biopolymer materials based on chitosan analysis and characterization [25]. Just before conducting the analysis, the chitosan samples are dissolved in distilled water and allowed to form an even suspension by stirring gently, so that an equal distribution of particles is achieved. The suspension is then placed in clear disposable zeta potential analysis cells and analyzed. Analysis is performed at an established temperature range of 25°C and uses water as the dispersant agent, and multiple analysis cycles are carried out for validation.

2.4 Rheological Measurement

Rheological studies have consistently shown that chitosan solutions exhibit non-Newtonian pseudoplastic behaviour, characterised by shear-thinning properties in which viscosity decreases with increasing shear rate due to the alignment and disentanglement of polymer chains under shear forces [25]. Previous findings indicate that higher chitosan concentration and molecular weight result in increased viscosity and consistency coefficient (K), alongside a reduced flow behaviour index ($n < 1$), confirming stronger chain entanglement and pseudoplastic flow behaviour [26]. This shear-dependent viscosity is particularly significant in aqueous and acidic environments, where increased concentration promotes the formation of transient polymer networks, further enhancing shear-thinning characteristics [26]. In wastewater treatment applications, this rheological behaviour is advantageous, as high viscosity at low shear rates supports effective floc formation through particle bridging, while reduced viscosity at high shear rates facilitates efficient dispersion and mixing of chitosan during rapid mixing stages [25][26]. This research was conducted by using Discovery HR-1 hybrid rheometer to measure the rheological measurement.

2.5 Yield Measurement

For the last test for chitosan characterization is yield calculation where it is to determine the efficiency of the extraction of the process. This process need weigh the dry chitin weight, initial mushroom powder weight, dry chitosan weight, and dry chitin weight. Every mass need to be recorded as it useful in testing process [22]. The calculation of the yield amount as the following formula :-

Chitin yield (%) :

$$\frac{Dry\ chitin\ weight}{Initial\ mushrom\ powder\ weight} \times 100\% \quad (3)$$

Chitosan yield (%) :

$$\frac{Dry\ chitosan\ weight}{Dry\ chitin\ weight} \times 100\% \quad (4)$$

This calculation will helps in comparison between the yield measurement between oyster and enoki mushrooms.

2.6 Wastewater Treatment performance Tests

The effectiveness of chitosan in treating wastewater is normally determined by a jar test experiment to determine its efficiency as a coagulant/flocculant agent in removing turbidity and suspension solids from the wastewater [27]. In a jar test experiment, a given amount of chitosan is added to a sample of actual wastewater obtained from a drain, followed by a rapid and slow agitation process to ensure a flocculation process [28]. The turbidity of the supernatant is then analyzed to determine the removal efficiency. Furthermore, the removal of turbidity can be quantitatively measured by a turbidity meter and qualitatively by observing floc formation and wastewater clarification to confirm the efficacy of the coagulation process [29]. Further, pH optimization involves carrying outJar tests at varying levels of pH to ascertain the optimal pH range for optimal chitosan performance. Chitosan performs better as a coagulant at an acidic to slightly alkaline environment due to its polycationic property influenced by variations in pH, which further determines its solubility properties to interact with particulates present in the wastewater [30].

3. Result and Discussion

3.1 Fourier Transform Infrared Spectroscopy (FTIR)

In general, FTIR spectroscopy was able to verify that all chitosan extracts contained the characteristic functional groups in chitosan in the form of O-H and N-H bonds, as well as amide I bands, meaning that chitin was successfully changed into chitosan from both enoki and oyster mushrooms [31]. The appearance of nearly identical FTIR spectra for all samples indicates that their structures are fairly similar. In the 1% solutions of chitosan, there was a reduction in or elimination of the peak for C-N stretching (amide III), likely because of dilution effects that cause a decrease in absorbance intensity beyond detection capacity for the FTIR equipment. It is also likely because of peak overlap [32]. It is not an indication of degradation of chitosan. The degree of deacetylation (DDA) level of all samples was determined to be comparable and of relatively high values. The main reason for that was that the DDA measurements are based on the absorbance ratio, not peak intensity, and after attaining a high degree of deacetylation, small variations in the absorbance values will not lead to variations in the degree of deacetylation. Based on Figure 2, stock enoki mushroom chitosan exhibits three characteristic FTIR peaks at 3255.83 cm-1, 1636.70 cm-1, and 1275.08 cm-1, corresponding to O–H/N–H stretching, amide I (C=O stretching), and C–N stretching, respectively. These peaks confirm the successful conversion of chitin into chitosan. The calculated degree of deacetylation (DDA) for this sample is 84.44%, indicating a high proportion of free amine groups. For 1% stock enoki mushroom chitosan in Figure 3 only two dominant peaks were detected at 3244.65 cm-1 and 1636.43 cm-1, while the C–N stretching peak was undetected. This reduction in peak number may be due to dilution effects, where lower concentration results in weaker absorbance intensity. However, the DDA value remains high at 84.72%, suggesting that dilution does not significantly affect the chemical structure of chitosan. Similarly, stock oyster mushroom chitosan (Figure 4) shows three clear peaks at 3255.83 cm-1, 1636.49 cm-1, and 1276.61 cm-1, with a DDA of 84.74%. In contrast, 1% stock oyster mushroom chitosan (Figure 5) exhibits only two detectable peaks, indicating reduced signal intensity at lower concentration. Overall, the FTIR results confirm that both enoki and oyster mushrooms successfully produced chitosan with high DDA values. The presence of O-H and N-H functional groups explains the strong positive surface charge and effective coagulation performance observed in later tests.

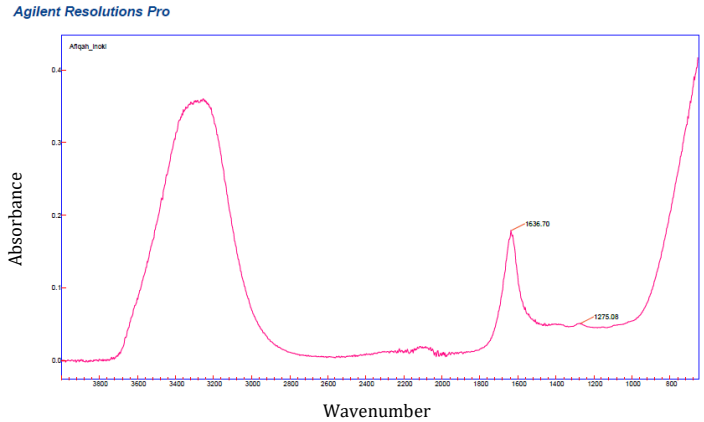

Fig. 2 *FTIR spectrum of stock chitosan derived from enoki mushroom*

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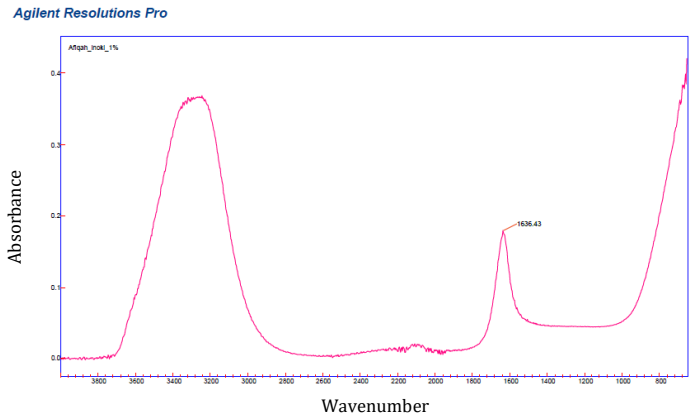

Fig. 3 *FTIR spectrum of 1% stock chitosan derived from enoki mushroom*

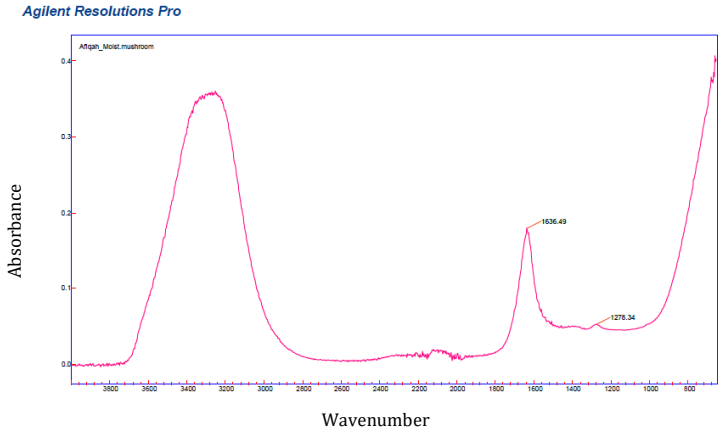

Fig. 4 *FTIR spectrum of stock chitosan derived from oyster mushroom*

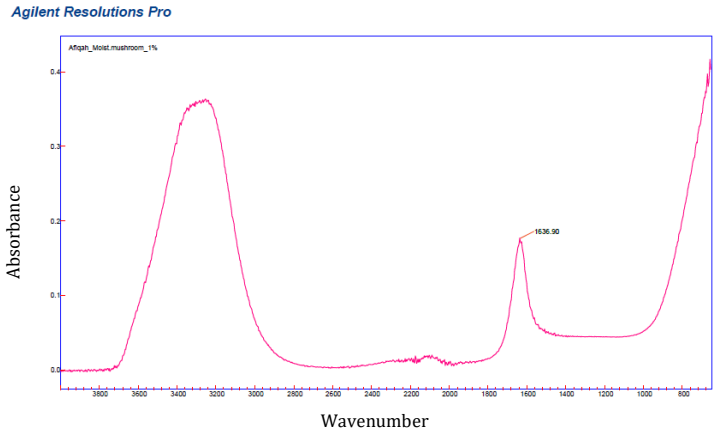

Fig. 5 *FTIR spectrum of stock chitosan derived from 1% oyster mushroom*

3.2 Zeta-Potential Analyzer test

Zeta potential analysis indicated that all the chitosan samples possessed positive surface charges. The zeta potential values for enoki mushroom chitosan were higher than those for oyster mushroom chitosan, indicating higher electrostatic interactions for the enoki mushroom chitosan towards the negative suspended particulate matter in the wastewater [33]. This is an indication that the enoki mushroom chitosan has a higher concentration of protonated amine groups. Zeta potential results show that all chitosan samples possess a positive surface charge, which is essential for destabilising negatively charged suspended particles. As shown in Figure 6, stock enoki mushroom chitosan recorded a high zeta potential value of +71.5 mV, indicating strong electrostatic attraction capability. Interestingly, 1% stock enoki mushroom chitosan in Figure 7 shows an even higher zeta potential value of +77.5 mV. This may be due to better dispersion of polymer chains at lower concentration, allowing more amine groups to be exposed in solution. This result suggests that surface charge is not solely

dependent on concentration but also on molecular arrangement. For oyster mushroom chitosan, stock solution recorded a zeta potential of +50.3 mV in Figure 8 while 1% stock solution showed a slightly lower value of +47.8 mV with higher deviation in Figure 9. The higher deviation indicates less stable charge distribution, which may explain the inconsistent coagulation performance observed during jar tests. Comparatively, enoki mushroom chitosan exhibits stronger and more stable positive charge than oyster mushroom chitosan, supporting its better performance under neutral pH conditions.

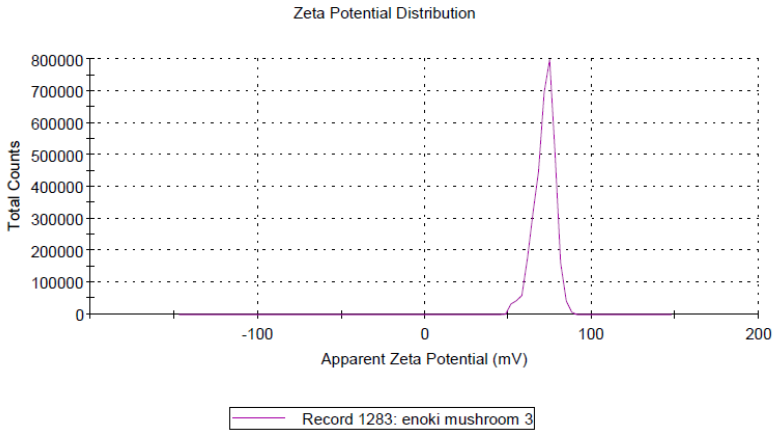

Fig. 6 *Zeta-Potential Analysis Test spectrum of stock chitosan derived from enoki mushroom*

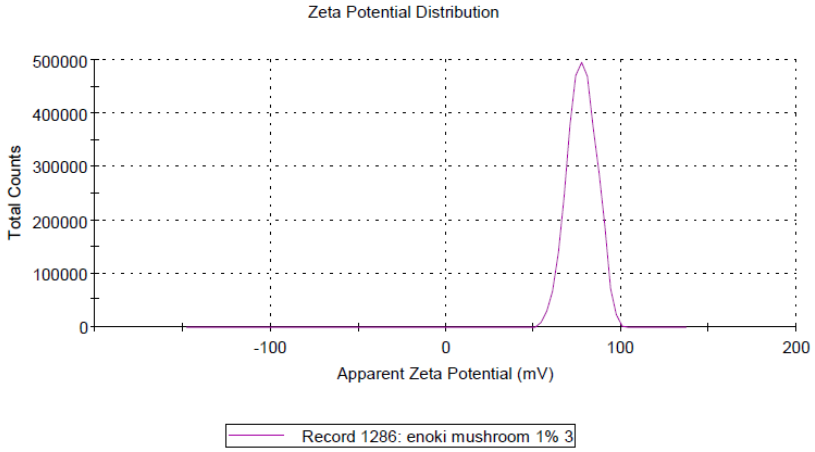

Fig. 7 *Zeta-Potential Analysis Test spectrum of 1% stock chitosan derived from enoki* mushroom

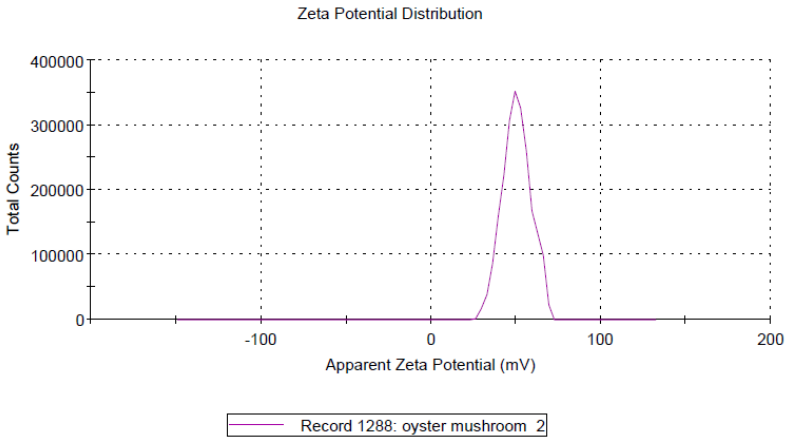

Fig. 8 *Zeta-Potential Analysis Test spectrum of stock chitosan derived from oyster mushroom*

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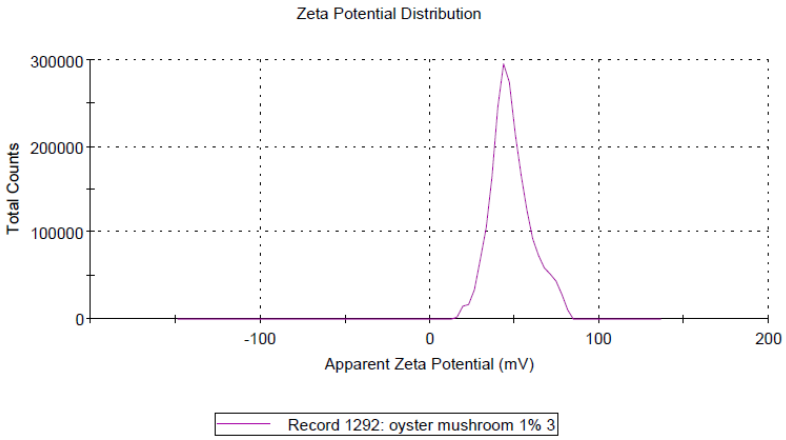

Fig. 9 *Zeta-Potential Analysis Test spectrum of 1% stock chitosan derived from oyster mushroom*

3.3 Rheological Measurement

Rheological analysis indicated that all chitosan solutions are shear-thinning fluids, in which viscosity reduces with the increase in shear rate. This property can be regarded as ideal in coagulation. At low shear rates, the increase in viscosity helps in the formation of polymer chains and flocs, whereas, at higher shear rates, lower viscosity helps in their better mixability [34]. Some viscosity data, especially under high shear rates, may seem implausible. Under low shear rates, the sensitivity of the equipment may contribute to erratic data. Under high shear rates, the alignment of polymer chains, wall slip, and degradation of the polymer structure may lead to low viscosity [35]. The differences in chitosan extracts from enoki and oyster mushrooms may be explained by molecular weight and the ability of the polymer chains. In diluted chitosan solutions (1%), the viscosity was always lower because of less intermolecular interactions, and this is as expected. Alum solutions can be classified as Newtonian fluids with low viscosity. They neither contribute to the strength of flocs by bridging nor participate in the process of floc formation. But, the non-Newtonian nature of chitosan increases the size of flocs and thus offers a greater advantage over other materials [36].

Rheological results (Figures 10 - 13) show that all chitosan samples exhibit shear-thinning behaviour. For stock enoki mushroom chitosan (Figure 10), viscosity decreases significantly from 3.26 Pa·s at low shear rate to 0.19 Pa·s at higher shear rate. This behaviour indicates strong polymer chain entanglement that becomes aligned under shear. In contrast, 1% stock enoki mushroom chitosan (Figure 11) shows much lower viscosity values, decreasing from approximately 0.0145 Pa·s to 0.0035 Pa·s. This confirms that dilution reduces intermolecular interactions, resulting in lower resistance to flow. For oyster mushroom chitosan, stock solution viscosity decreases from 0.56 Pa·s to 0.0076 Pa·s (Figure 12), while 1% solution decreases from 0.0295 Pa·s to 0.0033 Pa·s (Figure 13). These results indicate that enoki mushroom chitosan has higher viscosity compared to oyster mushroom chitosan, which may enhance floc formation through polymer bridging during coagulation.

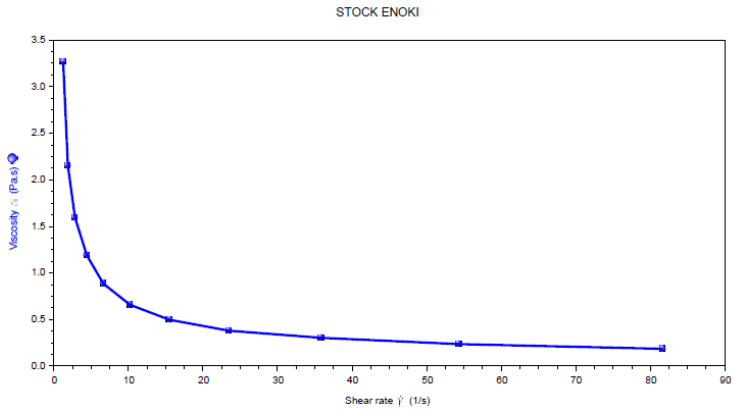

Fig. 10 *Rheometer spectrum of Viscosity as a function of shear rate for stock enoki mushroom chitosan solutions*

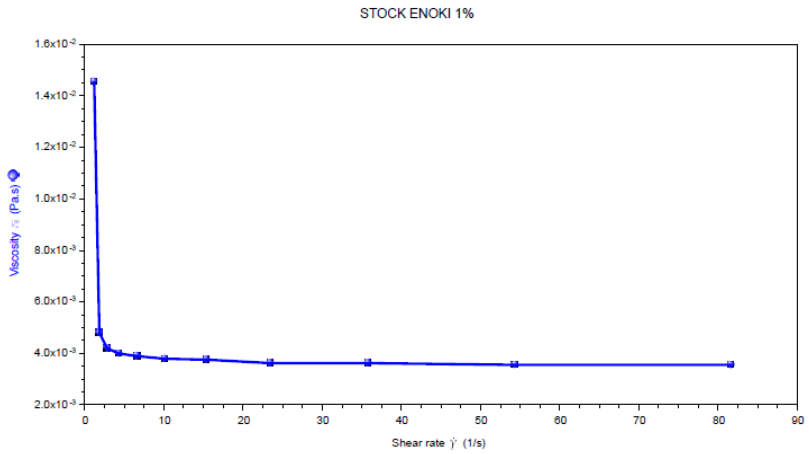

Fig. 11 *Rheometer spectrum of Viscosity as a function of shear rate for 1% stock enoki mushroom chitosan solutions*

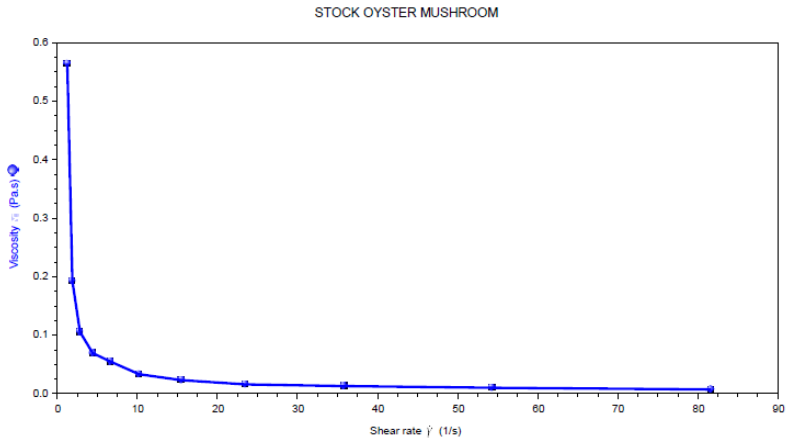

Fig. 12 *Rheometer spectrum of Viscosity as a function of shear rate for stock oyster mushroom chitosan solutions*

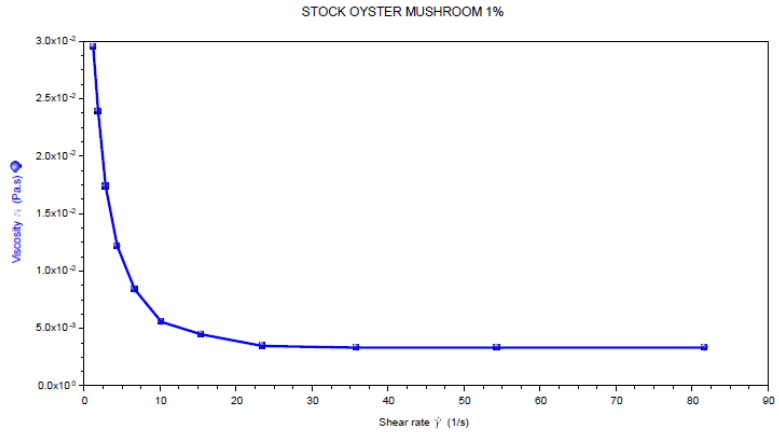

Fig. 13 *Rheometer spectrum of Viscosity as a function of shear rate for 1% stock oyster mushroom chitosan solutions*

3.4 Yield Measurement

The result of extraction demonstrated that there was a significant difference in the yield of chitosan between enoki mushroom (*Flammulina filiformis*) and oyster mushroom (*Pleurotus ostreatus*). Enoki mushroom yielded a

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significantly higher quantity of chitosan compared to oyster mushroom. This result suggests that enoki mushroom has a higher effective chitin content or a better cell wall structure that can easily respond to the chemicals in the deproteinization, demineralization, and deacetylation steps.

The yield of the oyster mushroom seems unusual or even abnormal at first. However, there could be a number of reasons for it. In oyster mushrooms, the lignocellulosic content of the cells in their walls appears to be higher, resulting in the incomplete penetration of the alkaline and the acidic solutions, and hence the incomplete extraction of the chitin. Moreover, the losses occurring during the different steps of the process of washing, filtration, and drying could also cause the lowering of the yield. This is owing to the sensitivity of the chitosan extraction process to temperature and reaction time. Figure 5 shows the trend of yield measurement for both Enoki & Oyster mushroom chitosan powder where the chitosan powder yield for enoki mushroom species is 18.09% meanwhile 1.33% for oyster mushroom species.

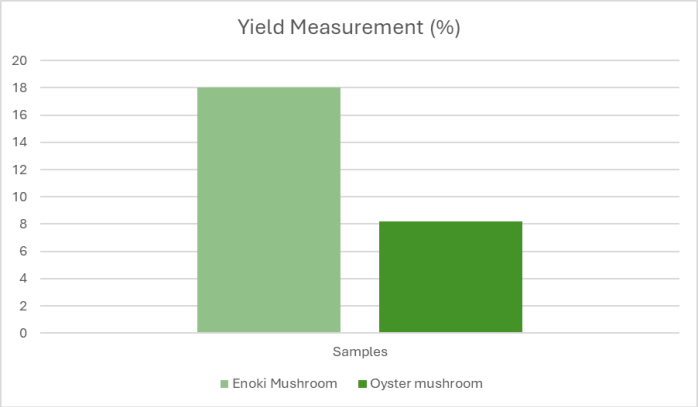

Fig. 5 *Yield Chitosan Measurement of both Enoki & Oyster Mushroom Powder*.

3.5 Wastewater Treatment performance Tests

As revealed by the results obtained using the jar test, the efficiency of coagulation for fungal chitosan is dependent on pH values, as these values have a significant effect on the protonation step that destabilizes the coagulated particles. The result showed that the coagulation process using Enoki mushroom chitosan significantly removed the turbidity of the coagulated particles. This is consistent with previous research, which suggested that optimal coagulation efficiency for chitosan can only be obtained in strongly acidic conditions, as the electrostatic attraction between the coagulant and negatively charged coagulated particles is highest during such conditions [16][37]. At pH 7, turbidity removal in enoki chitosan remained relatively constant, while in oyster chitosan, it was relatively more inconsistent, implying better charge density and bridging properties in enoki chitosan. A similar phenomenon also occurred, where chitosan was active at near-neutral pH but was dosage-dependent and susceptible to the method of mixing [38].

At pH 7.5, the turbidity increased because of the decreased protonation of chitosan in the basic pH solution. Moreover, previous studies have shown that chitosan had less coagulation efficiency at higher pH values [39]. Although alum is effective in the broad pH range, the production of higher amounts of sludge with the possibility of aluminium ions' retention in the water is a drawback. Conversely, chitosan is biodegradable with no presence of metal ions in the water. At pH 6 in Table 2, significant turbidity reduction was observed for both fungal-derived chitosan and alum. Stock oyster mushroom chitosan showed strong performance, achieving final turbidity of 21.6 NTU, which is comparable to alum (22.5 NTU). This confirms that acidic conditions favour chitosan coagulation due to higher protonation of amine groups. At pH 7 in Table 3, stock enoki mushroom chitosan achieved the lowest final turbidity of 33 NTU, outperforming alum (40 NTU). This indicates that enoki mushroom chitosan is more effective under neutral pH, which aligns with its higher zeta potential stability. However, at pH 7.5 Table 4, coagulation performance declined significantly, especially for diluted chitosan samples. This is due to reduced protonation of amine groups under alkaline conditions, resulting in weaker charge neutralisation and poor floc formation.

Table 2 Parameters of measurement for Jar test pH 6.

Type of coagulant	Beakers or jars					
	Blank	1	2	3	4	5
		Alum		1% Stock Enoki		1% Stock Oyster

			Stock Enoki mushroom chitosan	mushroom chitosan	Stock Oyster mushroom chitosan	mushroom chitosan
Volume of coagulant, mL	2	2	2	2	2	2
Initial pH	6	6	6	6	6	6
Initial turbidity,NTU	53.5	53.5	53.5	53.5	53.5	53.5
Initial temperature,°C	23.1	23.1	23.1	23.1	23.1	23.1
Final pH	5.20	4.31	4.76	5.17	4.61	5.10
Final turbidity, NTU	53.5	22.5	59.7	101	21.6	41.7
Final temperature, °C	23.1	22.8	22.5	22.5	22.3	22.0
Depth of coagulant (cm)	0.8	1.8	1.2	1.2	2.0	1.3

Table 3 Parameters of measurement for Jar test pH 7.

Type of coagulant	Beakers or jars					
	Blank	1 Alum	2 Stock Enoki mushroom chitosan	3 1% Stock Enoki mushroom chitosan	4 Stock Oyster mushroom chitosan	5 1% Stock Oyster mushroom chitosan
Volume of coagulant, mL	3	3	3	3	3	3
Initial pH	7	7	7	7	7	7
Initial turbidity,NTU	800	800	800	800	800	800
Initial temperature,°C	22.1	22.1	22.1	22.1	22.1	22.1
Final pH	6.73	5.15	5.76	6.88	5.75	6.72
Final turbidity, NTU	800	40.0	33.0	750	700	750
Final temperature, °C	22.1	22.1	22.1	22.6	22.5	22.1
Depth of coagulant (cm)	0.3	1.6	2.0	0.4	0.8	0.4

Table 4 Parameters of measurement for Jar test pH 7.5.

Type of coagulant	Beakers or jars					
	Blank	1 Alum	2 Stock Enoki mushroom chitosan	3 1% Stock Enoki mushroom chitosan	4 Stock Oyster mushroom chitosan	5 1% Stock Oyster mushroom chitosan
Volume of coagulant, mL	3	3	3	3	3	3
Initial pH	7.5	7.5	7.5	7.5	7.5	7.5
Initial turbidity,NTU	40	40	40	40	40	40
Initial temperature,°C	21.7	21.7	21.7	21.7	21.7	21.7
Final pH	6.82	5.53	5.98	6.65	5.84	6.86
Final turbidity, NTU	40.0	20.9	39.5	2000	60	1300
Final temperature, °C	21.7	21.8	21.7	21.7	21.9	21.7
Depth of coagulant (cm)	0	2.1	1.0	0.7	0.2	0.1

4. Conclusion

All the research objectives were accomplished successfully. Chitosan extraction using the chemicals deproteinization, acid hydrolysis, decolorization, deacetylation, and homogenization of the enoki mushroom (*Flammulina filiformis*) and the oyster mushroom (*Pleurotus ostreatus*) was accomplished successfully. Also, chitosan physical and chemical characterizations using FTIR, zeta potential, rheology measurement, and yield were carried out successfully. Enoki chitosan has positive charge (+70 mV) and viscosity values (ranging from 10^{0} Pa.s to 10^{-1}-10^{-3} Pa.s when shear rates increase) higher than the oyster chitosan. The effectiveness of chitosan obtained from mushrooms as a bio-coagulant for wastewater treatment has been ascertained. The results show that chitosan from the Enoki mushroom is superior to others in its ability to act as a bio-coagulant because of its higher charge and viscosity; however, oyster mushroom chitosan was found to be inferior to other type of mushooms in its properties and functions. The study shows that it is better to use Enoki mushrooms than others for obtaining chitosan for water treatment purposes.

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Conflict of Interest

Authors declare that there is no conflict regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** Nur Afiqah Sahira Saiful, Nor Faizah Razali; ***data collection:*** Nur Afiqah Sahira Saiful, Nor Faizah Razali; ***analysis and interpretation of results:*** Nur Afiqah Sahira Saiful, Nor Faizah Razali; ***draft manuscript preparation*****:** Nur Afiqah Sahira Saiful, Nor Faizah Razali reviewed the results and approved the final version of the manuscript.

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