

Evaluating the Efficiency of Toman Fish (*Channa micropeltes*) Chitosan in Reducing COD and Other Pollutants in Tofu Wastewater

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Abstract Wastewater from the tofu industry is a major source of environmental pollution in Indonesia due to its high organic content, which contributes to increased Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), and turbidity. Many small-scale tofu producers do not have adequate wastewater treatment systems due to limited access to affordable and environmentally friendly technology. This study evaluated the effectiveness of chitosan extracted from snakehead fish (*Channa micropeltes*) as a natural coagulant to improve the physicochemical quality of tofu wastewater. Chitosan was produced from fish skin and scales through chemical deacetylation and applied at three doses (20, 24, and 28 mg/L). The parameters evaluated included COD, BOD₅, TSS, turbidity, and pH. The results showed that the application of chitosan reduced the COD and BOD levels at all doses tested, with COD showing a statistically significant difference between treatments ($p < 0.05$). A decrease in TSS was observed descriptively but was not statistically supported. Conversely, turbidity increased after chitosan addition, indicating the presence of fine

suspended particles after the coagulation–flocculation process. The pH of the treated wastewater remained within the acidic range and did not approach neutral conditions. Overall, these findings indicate that chitosan derived from *Channa micropeltes* has potential as a natural coagulant for reducing organic pollution in tofu wastewater, particularly COD. However, additional treatment steps and optimization of operational conditions are required to address turbidity and pH adjustment. The utilization of fishery waste as a chitosan source also supports sustainable wastewater management and circular economy practices.

Keywords Chitosan, *Channa micropeltes*, Tofu Waste, Wastewater Treatment

1. Introduction

The tofu processing industry is a rapidly growing food sector in various regions of Indonesia due to high public demand for soy-based food products. However, despite its

simple production and high economic value, this industry produces large amounts of liquid waste, which is generally discharged directly into the environment without adequate treatment [1, 2]. Tofu wastewater contains high concentrations of organic matter, including compounds that can increase Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) values, and cause serious water pollution [3-5]. If left unchecked, this can degrade the quality of water bodies, disrupt aquatic life, and negatively impact public health.

Efforts to treat tofu waste have been hampered by high costs, particularly when using conventional, environmentally unfriendly chemical coagulants. This context has created a need for alternative solutions that are more sustainable, affordable, and environmentally safe. One approach that is gaining attention is the use of natural, biopolymer-based coagulants such as chitosan, known for its ability to purify water through a coagulation-flocculation process [6, 7].

Most of the research on chitosan so far has used shrimp or crab waste as the basic material [8, 9]. However, the potential of locally available freshwater fish, such as *Channa micropeltes*, remains underexplored as a source of chitosan for wastewater treatment. This fish has skin and scales rich in chitin, which can be processed into chitosan with similar effectiveness [10], and even has the potential to become a more affordable alternative in inland areas or areas with abundant freshwater fisheries.

Based on this background, this study aims to evaluate the effectiveness of chitosan extracted from snakehead fish in reducing COD levels and other pollutant parameters such as TSS, turbidity, BOD, and pH in tofu wastewater. Therefore, this research is expected to provide scientific contributions to the field of domestic wastewater treatment and support circular economy practices through the utilization of fishery waste.

2. Materials and Methods

2.1. Research Design and Location

This study used a laboratory-based experimental design using a completely randomized design (CRD) to evaluate the effectiveness of chitosan derived from snakehead fish (*Channa micropeltes*) in reducing pollutant parameters in tofu wastewater. The parameters tested included Total Suspended Solids (TSS), pH, turbidity, Biological Oxygen Demand (BOD), which was determined as BOD following a 5-day incubation period, and Chemical Oxygen Demand (COD). The study was conducted at the Water Quality and Hydro-Bioecology Laboratory, Faculty of Fisheries, Lambung Mangkurat University, using tofu waste collected from a local tofu industry in Banjarbaru City. This research has obtained ethical approval with the number 410/UMB/KE/VI/2025 on June 13, 2025.

2.2. Materials and Apparatus

The main materials in this study were tofu liquid waste and chitosan extracted from snakehead fish, specifically its scales and skin. The tofu waste was collected fresh from a tofu factory with a total volume of approximately 20 liters. The chemicals used in the extraction and analysis process included NaOH, HCl, and technical ethanol solutions, as well as reagents for BOD and COD measurements according to APHA standard methods. Laboratory apparatus and equipment included a digital pH meter, turbidimeter, dissolved oxygen (DO) meter, jar test apparatus, BOD incubator, COD reflux apparatus, oven, and analytical balance.

Chitosan Extraction Procedure from Snakehead Fish

Chitosan was extracted from snakehead fish waste using a sequential chemical method. The first step was deproteinization, which involved soaking the fish scales and skin in a 4% NaOH solution at 90 °C for 2 hours to remove protein. Demineralization was then carried out by soaking the residue in a 1N HCl solution for 2 hours to remove mineral content. After that, decolorization was carried out using 95% ethanol to remove pigments and color compounds. The final step was deacetylation, which involved heating chitin in a 50% NaOH solution at 100 °C for 3 hours to produce chitosan. The chitosan formed was then washed until the pH was neutral and dried in an oven at 50 °C for 24 hours.

2.3. Wastewater Treatment and Testing

After the chitosan was obtained, tofu waste samples in 1-liter containers were treated with three variations of chitosan dosages: 20 mg/L, 24 mg/L, and 28 mg/L, each with three replications. The samples were stirred using a jar tester at a fast speed (120 rpm) for 2 minutes, followed by a low speed (30 rpm) for 20 minutes, and then left for 1 hour for the sedimentation process. After the coagulation and flocculation processes were completed, the upper part (supernatant) of each sample was taken for analysis.

TSS parameter was measured using the gravimetric method, pH was measured using a calibrated digital pH meter, turbidity was measured using a turbidimeter, while BOD was analyzed using the Winkler method with a 5-day incubation at 20 °C. For COD determination, the colorimetric method with closed reflux (APHA Standard Method 5220 D) was used.

2.4. Data Analysis

The measurement data were analyzed using analysis of variance (one-way ANOVA) to determine the significant effect of chitosan dosage on each water quality parameter. If a significant difference was found, a Least Significant Difference (LSD) test was used to determine the differences between treatments. All analyses were performed at a significance level of $p < 0.05$ using IBM

SPSS Statistics software version 25 [11]. Results are presented as mean \pm standard deviation.

3. Result

Table 1 presents the physicochemical characteristics of tofu wastewater after treatment with chitosan derived from snakehead fish (*Channa micropeltes*) at three dosage levels: 20, 24 and 28 mg/L. COD concentrations decreased markedly after chitosan application at all tested dosages, with the lowest average COD observed at 24 mg/L. One-way ANOVA revealed a statistically significant difference among treatments ($p < 0.05$). BOD values also decreased substantially compared to the initial condition; however, statistical analysis did not provide sufficient evidence to confirm significant differences among dosages ($p > 0.05$). TSS concentrations decreased after treatment, particularly at a dosage of 20 mg/L, although the

differences among treatments were not statistically significant. In contrast, turbidity values increased following chitosan addition at all dosages compared to the initial condition. The pH of the wastewater remained acidic after treatment and did not approach neutral conditions. The effectiveness of chitosan as a coagulant is influenced by wastewater characteristics and operational conditions. Whereas changes in COD, TSS, turbidity, BOD and pH reflect the response of the wastewater to the applied treatment.

Figure 1 illustrates the variation of physicochemical parameters of tofu wastewater at different dosages of chitosan derived from *Channa micropeltes* (20, 24, and 28 mg/L). Differences in BOD and COD values are observed among the tested dosages, with relatively small variations across treatments. Similar variations are also observed for TSS, while turbidity values remain high after treatment. The pH values show only minor changes and remain within the acidic range.

Table 1. Response variables of TSS, pH, turbidity, BOD, and COD parameters to Toman Fish chitosan dosage (20 mg, 24 mg and 28 mg)

Parameters	Repetition	Chitosan Mass			
		Initial Test	20 mg	24 mg	28 mg
TSS (mg/L)		816			
	1		576	583	598
	2		559	633	624
	3		578	602	607
	Average		571	606	610
pH		3.83			
	1		2.01	3.01	2.00
	2		2.00	3.01	2.02
	3		2.02	3.00	2.01
	Average		2.01	3.01	2.01
Turbidity (NTU)		739			
	1		1406	1297	1407
	2		1234	1346	1148
	3		1190	1398	1101
	Average		1277	1347	1219
BOD (mgO/L)		9314.90			
	1		111.71	85.59	83.78
	2		97.30	61.26	81.08
	3		84.68	58.56	81.08
	Average		97.90	68.47	81.98
COD (mgO/L)		13516.60			
	1		188.14	188.14	203.19
	2		191.90	188.14	210.71
	3		188.14	188.14	203.19
	Average		189.39	188.14	205.70

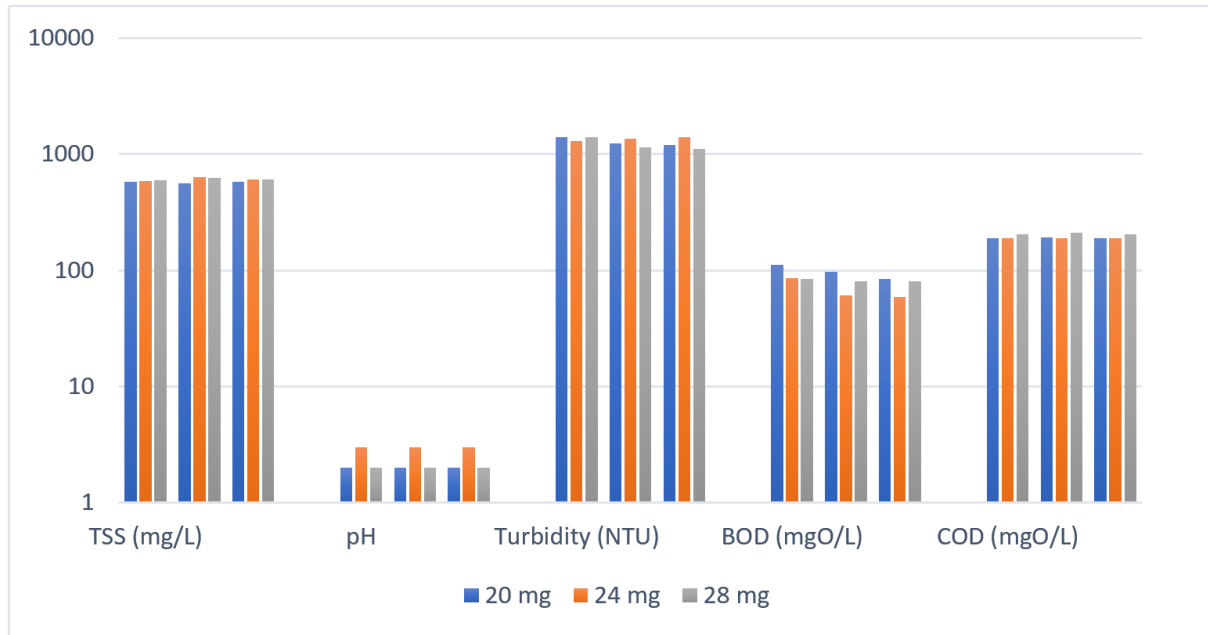


Figure 1. Changes in TSS, pH, turbidity, BOD, and COD of Tofu Wastewater After Chitosan Treatment

Table 2. Analysis of Variance of Water Parameters for each Chitosan Mass

Parameter	Source of Variation	df	MS	F	p-value	Fcrit	ANOVA Results Interpretation
TSS	Between Groups	2	1367	4.46	0.065	5.14	There is a tendency toward differences among treatments, but the differences are not statistically supported.
	Within Groups	6	307				
Turbidity	Between Groups	2	12390	0.87	0.466	5.14	The differences among treatments are not statistically significant.
	Within Groups	6	14245				
BOD	Between Groups	2	651	4.80	0.057	5.14	A tendency toward differences is observed; however, the differences are not statistically significant.
	Within Groups	6	136				
COD	Between Groups	2	288	36.66	< 0.001	5.14	Differences among treatments are statistically significant.
	Within Groups	6	8				

Table 2 presents the results of the one-way ANOVA analysis for TSS, turbidity, BOD, and COD parameters, including the source of variation, degrees of freedom (df), mean square (MS), F values, and p-values. The analysis shows that COD exhibited clear differences among treatment groups ($F = 36.643$, $p\text{-value} < 0.001$). Post hoc LSD analysis further indicated significant differences between the 20 mg/L and 28 mg/L groups as well as between the 24 mg/L and 28 mg/L groups, while no difference was observed between the 20 mg/L and 24 mg/L groups ($p = 0.604$). TSS and turbidity parameters did not show statistically supported differences among chitosan dosages, with p-values of 0.065 and 0.466, respectively. BOD showed a tendency toward differences among treatments ($F = 4.798$, $p\text{-value} = 0.057$); however, the differences were not statistically supported. The ANOVA results indicate that variations in chitosan dosage primarily affected COD reduction, while other parameters exhibited limited or inconsistent responses. The pH parameter is not

included in the one-way ANOVA analysis due to very limited variability within the treatment groups. As a result, the statistical assumptions required for reliable ANOVA interpretation could not be fully met. Therefore, pH changes are discussed descriptively.

4. Discussion

4.1. Chemical Oxygen Demand (COD)

The results showed that the administration of chitosan from snakehead fish significantly reduced COD levels in tofu wastewater ($p < 0.001$). This decrease reflects chitosan's effectiveness as a natural coagulant in the coagulation-flocculation process, where organic particles bind and form easily settleable flocs. This mechanism allows for efficient reduction of organic load, although not all doses showed a consistent downward trend.

These findings align with research by Arfi and Muflaha [12] that used chitosan from tilapia fish scales (*Oreochromis mossambicus*) as a coagulant in the treatment of vehicle wash wastewater. The study also showed a significant reduction in COD with varying optimal doses. The effectiveness of fish-derived chitosan in reducing COD levels confirms that fish waste-based biomaterials have significant potential as environmentally friendly and efficient wastewater treatment agents.

The effectiveness of chitosan in reducing COD levels, as shown in Figure 1, was optimal at doses of 20 mg/L and 24 mg/L, with relatively consistent results. Conversely, at a dose of 28 mg/L, COD levels increased, indicating that excessive use actually reduces coagulation efficiency. This finding is consistent with research by Arfi and Muflaha [12], where increasing the dose of chitosan from tilapia fish scales beyond the optimal point causes an excess cationic charge that disrupts the flocculation process and can trigger the resuspension of organic particles into the water. Therefore, the use of a lower dose actually shows higher efficiency per gram in reducing COD levels in wastewater.

All COD concentrations obtained in this study were below the maximum permissible limit of 300 mgO/L specified by the Indonesian Minister of Environment Regulation No. 5 of 2014, indicating compliance with applicable environmental quality standards.

4.2. Biochemical Oxygen Demand (BOD)

The BOD value in liquid tofu waste decreased after being treated with chitosan from snakehead fish (*Channa micropeltes*). The initial BOD was 9.314 mgO/L and then decreased to an average of 68.47 to 81.98 mgO/L at chitosan doses of 20, 24, and 28 mg/L (see Table 1). Descriptively, this indicates that chitosan has the ability to reduce the load of biodegradable organic pollutants. BOD values decreased after chitosan application at all tested doses, indicating a reduction in biodegradable organic matter in tofu wastewater. Although one-way ANOVA analysis showed a trend toward differences among chitosan doses (p -value = 0.057), this difference was not statistically supported. This pattern suggests that the coagulation-flocculation process facilitated by chitosan contributes to BOD reduction. However, varying the dose within the tested range did not produce a significantly different effect.

Although the reduction in BOD levels was not statistically supported, all treatments resulted in final BOD concentrations below the quality standard threshold stipulated in the Minister of Environment Regulation No. 5 of 2014 (150 mgO/L) for small-scale food processing industries. This indicates that the application of snakehead fish chitosan was effective in lowering BOD to levels that comply with existing environmental quality standards. However, compliance with discharge requirements should also consider other relevant wastewater parameters.

This finding is supported by research by Sela et al. [13] which used chitosan from shrimp shells in textile wastewater treatment. The study also reported that increasing the dose above the optimal point did not result in a statistically significant reduction in BOD, although there was a general decrease. This confirms that chitosan is more effective for physical and chemical parameters (such as COD and TSS), while less effective for biological parameters such as BOD. Its effectiveness may require additional treatment such as biofiltration or longer contact times.

4.3. Total Suspended Solid (TSS)

The TSS value in soybean curd wastewater decreased after treatment with chitosan from snakehead fish. The initial TSS value of 816 mg/L decreased to an average of 571 mg/L, 606 mg/L, and 610 mg/L at doses of 20, 24, and 28 mg/L, respectively. This decrease indicates that chitosan has the potential to be an effective natural coagulant in reducing suspended solids levels in wastewater. This decrease occurs because chitosan works as a natural coagulant that is able to neutralize the negative charge of suspended particles, thereby encouraging clumping (flocculation). The flocs formed are larger and heavier, so they easily settle to the bottom and directly reduce the TSS value in wastewater [14].

The ANOVA test results in Table 2 show $p = 0.065$, which indicates that variations in chitosan dosage did not have a statistically significant effect on reducing TSS, although the value approached the significance threshold (0.05). The 20 mg/L dosage actually showed the greatest reduction in TSS descriptively, while increasing the dosage to 24 mg/L and 28 mg/L did not produce a better reduction. This condition is likely caused by the administration of chitosan exceeding the optimal dosage, which can cause saturation of the cationic charge, thus disrupting the flocculation process and causing the formed flocs to become unstable or even redispersed (resuspended) into the solution.

These findings align with previous research by Nunez et al. [15] which examined the use of chitosan as an auxiliary coagulant in wastewater treatment. The study explained that after the optimal dose was reached, excessive addition of chitosan did not increase efficiency but instead caused a restabilization of suspended particles. As a result, the flocs formed became unstable and could be redispersed into the solution, reducing the effectiveness of the TSS sedimentation process and overall turbidity.

These findings confirm that the effectiveness of chitosan in reducing TSS is only optimal when used in certain doses. A dose of 20 mg/L provided the best reduction results descriptively, with an average TSS of 571 mg/L. Although not statistically significant, this value has successfully reduced TSS from the initial condition. And at this dose, the final TSS result has met the maximum TSS quality

standard of 200 mg/L, as stipulated in the Minister of Environment Regulation No. 5 of 2014 for small-scale tofu processing industries.

4.4. Turbidity

The results presented in Table 1 show that turbidity values increased after the addition of chitosan to tofu wastewater compared to the initial condition, rising from 739 NTU to 1277 NTU at 20 mg/L, 1347 NTU at 24 mg/L, and 1219 NTU at 28 mg/L. This increase may be associated with the presence of suspended chitosan particles, which are positively charged polymers that did not completely aggregate into stable flocs or settle effectively during the treatment process. These findings suggest that chitosan addition did not directly lead to a reduction in turbidity under the experimental conditions applied.

Previous studies have reported that the effect of chitosan on turbidity is strongly dependent on dosage. At appropriate dosages, chitosan can neutralize colloidal particle charges and promote the formation of settleable flocs. However, when the dosage is not optimal, chitosan may contribute to particle stabilization, hinder sedimentation, and result in increased turbidity. Therefore, careful optimization of chitosan dosage and operational conditions is essential to minimize turbidity and enhance overall treatment performance [16].

The ANOVA test in Table 2 shows $p = 0.466$, indicating that variations in chitosan dosage did not have a statistically significant effect on changes in turbidity. This result is in line with previous research which found that the molecular weight of chitosan did not have a significant effect on reducing turbidity, with a p -value of 0.2497. This means that even though chitosan was added as a coagulant agent, variations in its molecular weight were not strong enough to statistically affect the effectiveness of settling suspended particles [17].

The increase in turbidity after chitosan application indicates the need to optimize treatment conditions, including adjusting dosage and application methods. According to Indonesian Ministry of Environment Regulation No. 5 of 2014, wastewater turbidity must be at a level that does not pollute the environment, namely 25 NTU. Therefore, the use of chitosan needs to be combined with further treatment to ensure the final product meets quality standards and is safe for the aquatic environment.

4.5. Potential of Hydrogen (pH)

The pH values of tofu wastewater exhibited noticeable changes following treatment with chitosan derived from snakehead fish. The initial pH of the untreated wastewater was 3.83, indicating strongly acidic conditions. After chitosan application at dosages of 20, 24, and 28 mg/L, the average pH values were 2, 3, and 2, respectively. Overall,

the pH remained within the acidic range and did not show a clear shift toward neutral conditions.

The pH is an important parameter indicating the acidity or alkalinity of wastewater, which influences treatment efficiency and environmental safety. In this study, the average pH values of tofu wastewater after treatment with chitosan ranged between 2 and 3, indicating strongly acidic conditions. These values remain far below the recommended pH range for treated industrial wastewater (6–9), suggesting that the application of chitosan alone was insufficient to adjust the pH to environmentally acceptable levels.

The decrease in pH observed after treatment can be attributed to the chemical characteristics of chitosan. Chitosan contains amino functional groups that can undergo protonation and release hydrogen ions (H^+) in aqueous solutions, thereby lowering the pH. Similar acidic shifts following chitosan application have been reported in previous studies, particularly under conditions where pH adjustment or buffering was not applied prior to treatment [18].

Although chitosan effectively contributed to the reduction of organic pollution parameters, the persistently low pH indicates that additional treatment steps are required. Therefore, chitosan treatment should be considered as part of a multi-stage wastewater management process rather than a standalone solution for meeting pH-related environmental discharge standards [19, 20].

5. Conclusions

This study indicates that chitosan derived from snakehead fish (*Channa micropeltes*) has potential as a natural coagulant for tofu wastewater treatment, particularly in reducing organic pollutants as reflected by COD reduction. Variations in chitosan dosage affected COD values, while changes in TSS and BOD were not statistically supported. Turbidity increased after treatment, and the pH remained in the acidic range, indicating that additional treatment steps are required. Overall, fish-based chitosan shows promise as an environmentally friendly coagulant but should be applied as part of a multi-stage wastewater treatment process.

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