Chitosan as a Coagulant for Recovery of Proteinaceous Solids from Tofu Wastewater

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Chitosan was an effective coagulant for recovery of proteinaceous solids from tofu wastewater. Turbidity was reduced 97% by treatment with 300 mg/L chitosan at pH 5.8 (the original pH of tofu wastewater). In the control test, turbidity was reduced 30% after gravity settling for 1 h. Greater reductions in turbidity occurred with $Fe_2(SO_4)_3$ than with the other three inorganic salts tested. Combination of chitosan with $Fe_2(SO_4)_3$ did not further reduce turbidity. The relative effectiveness of treatment was studied with chitosans dissolved in various organic acids and with chitosan solutions stored at 4 °C for 0, 3, 6, or 12 days. The proximate and amino acid analyses of the coagulated solids revealed 41.9% protein with all essential amino acids, 1.5% fat, and 0.9% ash.

Keywords: *Chitosan; coagulant; tofu; wastewater*

INTRODUCTION

Tofu, made from soybeans, has long been consumed as a traditional food in Korea. As of 1992, 510 tofu plants were distributed throughout the country, producing approximately 300 000 tons yearly from around 100 000 tons of soybean (Anonymous, 1992). A significant volume of wastewater, with high chemical oxygen demand (COD) and proteinaceous solids, is discharged from the tofu preparation process. It is recognized that chitosan, with its partial positive charge, can be used for coagulation and recovery of proteinaceous solids present in such food-processing wastewater (Knorr, 1991).

Chitosan is a modified, natural, carbohydrate polymer derived by deacetylation of the naturally occurring chitin in the exoskeleton of crustacea such as crab, shrimp, and crawfish. Over the past several years, chitosan has been receiving increased attention for its applications in the chemical, biomedical, and food industries (Hirano and Tokura, 1982; Knorr, 1984, 1991; Muzzarelli, 1977; Muzzarelli and Pariser, 1978; No and Meyers, 1989; Skaugrud and Sargent, 1990; Skjak-Braek et al., 1988; Zikakis, 1984). The major commercial applications for chitosan currently are in industrial wastewater treatment and recovery of feed-grade material from food-processing plants. Since chitosan carries a partial positive charge, it effectively functions as a polycationic coagulating agent in wastewater treatment (Peniston and Johnson, 1970).

Several studies have demonstrated the effectiveness of chitosan for coagulation and recovery of suspended solids in processing wastes from poultry (Bough et al., 1975), eggs (Bough, 1975a), cheese (Bough and Landes, 1976; Wu et al., 1978), meat and fruit cakes (Bough, 1976), seafood (Bough, 1976; Johnson and Gallanger, 1984), and vegetable operations (Bough, 1975b; Moore et al., 1987), with reductions in suspended solids of 70–98% and COD of 55–80%. However, there is no available information on use of chitosan as a coagulant for treatment of tofu-processing wastewater.

The objective of the present research was to evaluate chitosan as a polyelectrolyte coagulant for coagulation and recovery of proteinaceous solids in wastewater discharged from tofu preparation processing plants.

EXPERIMENTAL PROCEDURES

Coagulants. A commercial chitosan (from crab shells, practical grade) used in this experiment was obtained from Keumho Chemical Products Co. (Seoul). The characteristics of chitosan were as follows: moisture, <10%; ash, <0.5%; viscosity of 0.5% chitosan solution in 1% acetic acid, 800–1000 cP; degree of deacetylation, >80%; molecular weight, 1.5×10^6.

Four inorganic salts used were ferric chloride ($FeCl_3{\cdot}6H_2O$), ferric sulfate [$Fe_2(SO_4)_3{\cdot}XH_2O$], calcium chloride ($CaCl_2$), and aluminum sulfate [$Al_2(SO_4)_3{\cdot}18H_2O$].

Preparation of Coagulant Solutions. Chitosan solutions (1% w/v) were prepared by dissolving chitosan in 1% acetic acid. The inorganic salts were dissolved in deionized water at a 1% (w/v) concentration. All solutions were freshly prepared before their application to wastewater.

Preparation of Tofu Wastewater. Tofu wastewater, obtained from coagulation and molding steps, was prepared in our laboratory, simulating general procedures used in the commercial tofu preparation process (Lee et al., 1990). Wastewater samples, prepared every 2 days, were placed in 2-L plastic containers and stored at 4 °C. Prior to use, the sample was allowed to reach room temperature.

Laboratory Jar Tests. The conventional jar test (Culp and Culp, 1971) was used to establish optimal conditions of pH and concentration of chitosan or inorganic salt. Wastewater samples (100 mL), adjusted to the desirable pH levels and appropriate concentrations of chitosan or inorganic salts, were stirred for 2 min at 100 rpm followed by 3 min at 30 rpm. In combination studies, chitosan and an inorganic salt coagulant solution were added sequentially to wastewater. After settling for 1 h at room temperature, the supernatant aliquots were withdrawn via a pipet. Turbidity in the supernatant was measured as nephelometric turbidity units (NTU) with a Hach 2100A turbidimeter.

Storage of Chitosan Solution. For evaluation of the coagulating ability of chitosan solutions with length of storage, 1% chitosan solutions in 1% acetic acid were stored at 4 °C for 0, 3, 6, and 12 days.

Recovery of Coagulated Solids. The coagulated solids from 2-L samples of wastewater were recovered by centrifugation at 3000 rpm for 20 min, dried in a forced-air oven for 3 h at 105 °C, and subjected to proximate and amino acid analyses.

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Table 1. Effect of pH and Chitosan Concentrations on Reduction of Turbidity[a] in Tofu Wastewater[b]

	concn of chitosan[c]				
pH	0 mg/L	200 mg/L	400 mg/L	600 mg/L	800 mg/L
4.0	236 ± 104^{aA}	145 ± 7^{bA}	184 ± 48^{bA}	145 ± 7^{bA}	151 ± 10^{bA}
5.0	302 ± 3^{aB}	18 ± 0^{bB}	6 ± 1.3^{cB}	713 ± 16^{bB}	542 ± 40^{eB}
5.8[c]	328 ± 14^{aC}	135 ± 0^{bA}	6 ± 0.3^{cB}	43 ± 20^{dC}	239 ± 38^{eC}
7.0	343 ± 5^{aD}	342 ± 151^{aC}	7 ± 1.3^{bB}	43 ± 8^{cD}	225 ± 143^{dD}
8.0	240 ± 57^{aE}	321 ± 62^{bC}	7 ± 0.3^{cB}	19 ± 6^{cE}	455 ± 7^{dE}

[a] Mean ± standard deviation of duplicate determinations. [b] Turbidity in raw tofu wastewater was 422 NTU. [c] Different lower case superscripts within a row indicate significant differences ($P < 0.05$). Different upper case superscripts within a column indicate significant differences ($P < 0.05$). [d] The original pH of tofu wastewater.

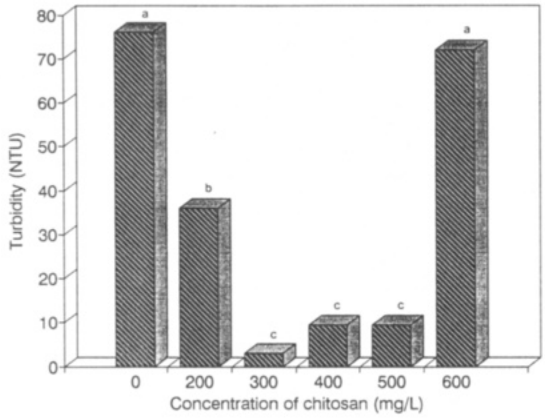

Figure 1. Effect of chitosan concentrations on reduction of turbidity in tofu wastewater at pH 5.8. Bars having the same letter above them are not significantly different ($P > 0.05$).

Proximate and Amino Acid Analyses. Protein content of dry coagulated solids was estimated from the sum of each amino acid content obtained by amino acid analysis. Moisture, ash, and fat were determined in duplicate by standard methods (AOAC, 1980). Amino acids were analyzed using an amino acid analyzer (Hitachi Model 835−50).

Statistical Analysis. The data were analyzed by analysis of variance. Means of the main effects were separated by Duncan's multiple-range test using the SAS software package (SAS, 1985).

RESULTS AND DISCUSSION

Optimum pH and Concentration of Chitosan. The effects of various pH values and concentrations of chitosan on reduction of turbidity in tofu wastewater are shown in Table 1. Results indicate that the most effective reduction of turbidity (6−7 NTU) was achieved by treatment with 400 mg/L chitosan at pH 5.0, 5.8, 7.0, and 8.0. Treatment with chitosan at pH 4.0 resulted in turbidity values greater than 145 NTU at all concentrations tested. These results demonstrate that the lowest turbidity can be achieved by treatment with the optimum concentration of chitosan at pH 5.8 (the original pH of tofu wastewater) without pH adjustment. Thus, all subsequent experiments were studied at the original pH (pH 5.8) of tofu wastewater. Several workers (Bough, 1975a; Johnson and Gallanger, 1984; Moore et al., 1987) have observed that chitosan was effective for coagulation of suspended solids in food-processing wastes at a similar pH level.

In an effort to further optimize the concentration of chitosan needed to obtain the lowest turbidity in tofu wastewater, chitosan was added at intervals of 100 mg/L from 200 to 600 mg/L at pH 5.8. Results are shown in Figure 1. Differences in turbidity values between Table 1 and Figure 1 are due to dissimilarity in initial turbidity values from batch to batch. The lowest turbidity was obtained with 300 mg/L chitosan, with reduction by 97% from 109 to 3.1 NTU. In the control test (without chitosan), turbidity was reduced by 30% from 109 to 76 NTU by gravity settling for 1 h. An increase in chitosan concentration from 500 to 600 mg/L resulted in a significant ($P < 0.05$) increase in turbidity values. This is probably due to restabilization of the colloidal suspension by excessive treatment of an organic polymer, as observed by Bough and Landes (1976) and Moore et al. (1987).

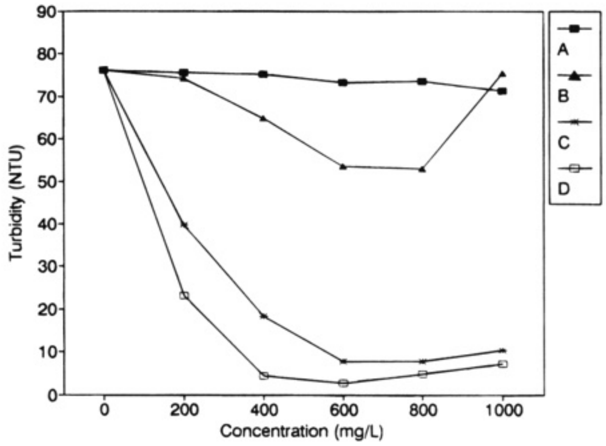

Figure 2. Effect of concentrations of four different inorganic salts on reduction of turbidity in tofu wastewater at pH 5.8. A, $CaCl_2$; B, $Al_2(SO_4)_3$; C, $FeCl_3$; D, $Fe_2(SO_4)_3$.

Comparison of Four Inorganic Salts as Coagulants. Four inorganic salts were compared to evaluate their ability to coagulate suspended solids in tofu wastewater at pH 5.8. Results (Figure 2) revealed greater reductions ($P < 0.05$) in turbidity with $FeCl_3$ and $Fe_2(SO_4)_3$ than with $CaCl_2$ and $Al_2(SO_4)_3$. This is similar to data on abrasive-peeled potato wastewater obtained by Karim and Sistrunk (1985). The greatest reductions in turbidity occurred with $Fe_2(SO_4)_3$ over all concentrations tested ($P < 0.05$). With 400 mg/L $Fe_2(SO_4)_3$, the turbidity value (NTU) of the wastewater was reduced from 109 to 4.4, representing a 96% reduction in turbidity of the original raw wastewater.

Combination of Chitosan with $Fe_2(SO_4)_3$. The combined effects of chitosan and $Fe_2(SO_4)_3$ were evaluated for greater reduction of turbidity in tofu wastewater (Table 2). The maximal reduction was obtained with 300 mg/L chitosan or 300 mg/L $Fe_2(SO_4)_3$ alone. Combination of chitosan with $Fe_2(SO_4)_3$ did not further reduce turbidity. Comparable results were observed by Bough et al. (1975) and No and Meyers (1989) on poultry chiller effluent and crawfish-processing wastewater, respectively. In some instances, however, effective reductions in turbidity were accomplished by combination of chitosan with a synthetic polyelectrolyte or an inorganic salt (Bough, 1975a, 1976; Bough and Landes, 1978).

Table 2. Effect of Combination of Chitosan with $Fe_2(SO_4)_3$ on Reduction of Turbidity in Tofu Wastewater at pH 5.8

coagulant	turbidity,[a] NTU
300 mg/L chitosan	3.8^{a}
200 mg/L chitosan + 100 mg/L $Fe_2(SO_4)_3$	10.4^{b}
100 mg/L chitosan + 200 mg/L $Fe_2(SO_4)_3$	159.0^{c}
300 mg/L $Fe_2(SO_4)_3$	5.1^{a}

[a] Average of duplicate determinations. Means with the same superscript are not significantly different ($P > 0.05$).

Reduction in turbidity with 300 mg/L $Fe_2(SO_4)_3$ was as effective as that with 300 mg/L chitosan. However, an advantage of chitosan, compared with $Fe_2(SO_4)_3$, is the absence of large levels of residual iron in the chitosan-treated supernatant and coagulated solids. Chitosan also has the advantage of being a nontoxic, biodegradable, colorless product. Furthermore, the intensely yellow supernatant, when treated with $Fe_2(SO_4)_3$, may need decoloration.

Effect of Different Chitosan Solvents. Various organic acids, used as a chitosan solvent, were compared with their possible effect on the coagulating ability of chitosan in treatment of tofu wastewater. One percent (w/v) chitosan solutions were all prepared in 1% organic acids. As seen in Table 3, formic acid was less effective in reduction of turbidity than were the other three organic acids, particularly at higher concentrations. Chitosans dissolved in acetic, lactic, or succinic acids appeared to show similar reductions in turbidity at equivalent concentrations. However, on statistical analysis, significant differences were observed among the three organic acids at specific concentrations. The lowest turbidity value (3.2 NTU) was obtained with lactic acid, followed by acetic acid (4.4 NTU) and succinic acid (4.9 NTU) at a concentration of 300 mg/L chitosan, the optimum concentration for treatment. Addition of 100 mg/L chitosan, irrespective of chitosan solvents, caused the raw wastewater to become more turbid, resulting in a significant ($P < 0.05$) increase in turbidity. This increased turbidity was readily observed visually. These results suggest that various solvents as well as concentrations of chitosan can affect the effectiveness of treatment. It should be noted that acetic acid is a commonly used organic acid for solubilizing chitosan (Bough, 1975a; Bough et al., 1975; No and Meyers, 1989).

Effect of Stored Chitosan Solution. Effectiveness of treatment was evaluated for the chitosan solutions stored at 4 °C for 0, 3, 6, or 12 days (Figure 3). All were applied at 300 mg/L, the most effective chitosan concentration. A chitosan solution stored for 3 days gave a reduction in turbidity (5.2 NTU) similar to that of the fresh one. However, chitosan solutions stored for 6 or 12 days showed lower coagulating ability, with turbidity (NTU) values of 14.7 and 14.9, respectively. Thus, it is recommended that chitosan solutions be freshly prepared if valid results are to be obtained. Further studies are needed to determine the coagulating ability of chitosan solutions with prolonged storage at room temperature, especially since decrease in viscosity with length of storage is a common feature of chitosan solutions (Moorjani et al., 1978). Lower coagulating ability and decrease in viscosity of chitosan solutions upon storage are probably due to aggregation of chitosan macromolecules and partial degradation of chitosan by acetic acid solutions.

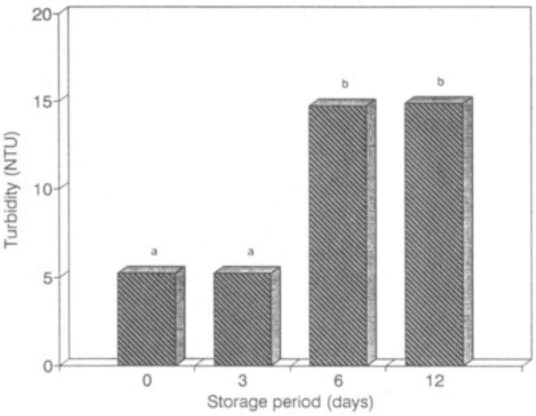

Figure 3. Effect of storage periods of chitosan solution on reduction of turbidity in tofu wastewater treated with 300 mg/L chitosan at pH 5.8. Bars having the same letter above them are not significantly different ($P > 0.05$).

Effect of Settling Time. Effect of settling time on turbidity of tofu wastewater treated with 300 mg/L chitosan at pH 5.8 is shown in Table 4. Coagulation and gravity settling for 0.5 h resulted in reduction of turbidity by 94% from 195 to 12.5 NTU. Further increase in gravity settling time gave a slightly but significantly increased reduction of turbidity. These data support earlier observations by Bough (1975a) and Bough et al. (1975) in egg-breaking wastewater and poultry-processing effluent, respectively. In contrast, No and Meyers (1989) found that coagulation and gravity settling for 0.5−3 h gave the same reduction of turbidity of crawfish-processing wastewater. Turbidity in food-processing wastes has been often reduced by 90% or more with coagulation followed by settling (Bough, 1975a,b; Johnson and Gallanger, 1984; Moore et al., 1987).

Analyses of Coagulated Solids. Proximate analysis of the coagulated solids (88.3% moisture) recovered by coagulation with 300 mg/L chitosan at pH 5.8 showed that average values for protein, fat, and ash were 41.9%, 1.5%, and 0.9%, respectively, on a dry basis.

The amino acid composition of the dry coagulated solids from tofu wastewater (Table 5) revealed relatively high contents of aspartic acid, serine, glycine, alanine,

Table 3. Effect of Concentrations of Chitosan Dissolved in Different Organic Acids on Reduction of Turbidity[a] in Tofu Wastewater[b] at pH 5.8

organic acid	concn of chitosan[c]					
	100 mg/L	200 mg/L	300 mg/L	400 mg/L	500 mg/L	600 mg/L
acetic	423 ± 30^{aA}	47.2 ± 3.6^{bA}	4.4 ± 1.0^{cA}	6.7 ± 0.3^{cA}	3.7 ± 0.3^{cA}	56.0 ± 12.7^{bA}
lactic	445 ± 7^{aB}	34.2 ± 1.8^{bB}	3.2 ± 0.3^{cB}	6.7 ± 0.3^{dA}	3.7 ± 0.3^{cA}	75.5 ± 9.2^{eB}
succinic	445 ± 7^{aB}	42.0 ± 8.5^{bC}	4.9 ± 0.1^{cC}	5.5 ± 0.7^{cA}	3.7 ± 0.3^{cA}	56.7 ± 9.5^{dA}
formic	405 ± 7^{aC}	13.7 ± 0.3^{bD}	11.5 ± 0.7^{bD}	59.5 ± 8.5^{cB}	555 ± 7.0^{dB}	500 ± 0.0^{eC}

[a] Mean ± standard deviation of duplicate determinations. [b] Turbidity in raw tofu wastewater was 125 NTU. [c] Different lower case superscripts within a row indicate significant differences ($P < 0.05$). Different upper case superscripts within a column indicate significant differences ($P < 0.05$).

Table 4. Effect of Settling Time on Turbidity of Tofu Wastewater Treated with 300 mg/L Chitosan at pH 5.8

sample	settling time, h	turbidity,[a] NTU	reduction of turbidity, %
raw	0	195.9^{a}	0
CS[b]	0.5	12.5^{b}	94
CS	1.0	8.0^{c}	96
CS	2.0	4.2^{c}	98
CS	3.0	1.0^{d}	99

[a] Average of duplicate determinations. Means with the same superscript are not significantly different ($P > 0.05$). [b] CS, coagulated and settled.

Table 5. Comparison of Amino Acid Composition of Dry Coagulated Solids from Tofu Wastewater and from Crawfish Wastewater

	content, mg/g	
amino acid[a]	tofu	crawfish[b]
aspartic acid	54.7	61.6
threonine*	18.5	21.1
serine	59.1	19.1
glutamic acid	12.1	121.3
glycine	56.7	17.2
alanine	46.1	43.0
cysteine	6.2	
valine*	22.8	22.7
methionine*	11.9	10.1
isoleucine*	2.4	13.7
leucine*	0.4	48.1
tyrosine	2.1	16.3
phenylalanine*	88.4	18.8
lysine*	9.6	35.5
histidine	17.9	8.1
arginine	3.0	43.5
proline	6.6	11.5
*essential amino acids	154.0	170.0
total	418.5	511.6

[a] Tryptophan was destroyed in the acid hydrolysis. [b] From No and Meyers (1989).

and phenylalanine. These five amino acids and seven essential amino acids accounted for 73% and 37%, respectively, of the total amino acids present in the coagulated solids. Differences can be seen in the amino acid composition of coagulated solids from tofu wastewater and that from a crawfish-processing operation (Table 5). The extremely high content of glutamic acid in the latter is especially noteworthy.

With such high contents of protein and essential amino acids and low ash content, the coagulated solids from tofu wastewater have potential as a nutritious ingredient in livestock feed formulations. The presence of small amounts of chitosan has no adverse effects on the nutritional value of proteins recovered from food-processing wastes (Bough and Landes, 1976). Chitosan has been reported to be safe and digestible and to have hypolipidemic activity for domestic animals (Hirano et al., 1990). The chitosan-coagulated solids may have wider application as feed additives than those containing inorganic salt coagulating agents in view of the absence of large levels of inorganics, especially iron and aluminum, and the approval for chitosan as a feed additive by the U.S. Food and Drug Administration (Knorr, 1984).

This study has demonstrated that chitosan is an effective coagulant for reduction and recovery of proteinaceous solids from tofu-processing wastewater. Turbidity was reduced 97% by treatment with 300 mg/L chitosan at pH 5.8. The coagulated solids have potential as valuable protein sources for livestock feeds. The total concentration of potentially recoverable compounds is quite significant in view of the magnitude of the total amount of water (about 10 times the amount of soybeans used) discharged from the tofu preparation process. The yield of dry coagulated solids could be as much as 4 kg/1000 L. The sale of byproducts as feed ingredients can partially offset wastewater treatment costs. However, the economics of waste treatment by coagulation and recovery of the coagulated solids should be examined. Further work is needed with various tofu wastewaters to adequately extrapolate the present data to commercial tofu preparation plants, especially since the level of proteinaceous solids present in the wastewater varies from plant to plant. Pilot-scale studies also are needed to evaluate methods and costs for dewatering and drying of coagulated solids. Further significance is seen for the use of chitosan as a tofu coagulant instead of the inorganic salts presently being used in commercial tofu plants.

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