Crawfish Chitosan as a Coagulant in Recovery of Organic Compounds from Seafood Processing Streams

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A chitosan, a cationic carbohydrate polymer, preparation from crawfish shell chitin coagulated suspended solids in crawfish pigment extraction process stickwater as effectively as, or better than, two commercial chitosans and five synthetic commercial polymers at pH 6.0. Concentration of suspended solids and turbidity were reduced 97% and 83%, respectively, by treatment with 150 mg/L chitosan at pH 6.0, with a 45% reduction in chemical oxygen demand (COD). In contrast, turbidity was reduced only 8% in the control test. Combinations of chitosan and $FeCl_3$ did not yield significant reduction in turbidity. The proximate composition of the coagulated solids, with 5.97 g/L yield, was 27.1% crude protein, 51.7% fat, and 3.3% ash. The supernatant revealed large concentrations of flavor-related free amino acids, including arginine, alanine, glutamic acid, serine, and glycine.

A significant concentration of potentially recoverable organics is present in discharge streams from shrimp processing plants in Louisiana (Meyers and Rutledge, 1973; Perkins and Meyers, 1977) as well as from the crawfish pigment extraction process itself (Chen and Meyers, 1982). This latter, organically rich, effluent is characterized by high chemical oxygen demand (COD), or biochemical oxygen demand, and total suspended solids. Effective recovery of organic compounds present in such discharges is realistic in terms of their potential utilization, as well as eventual reductions that can be achieved in effluent discharge loads.

Chitosan is a modified, natural, carbohydrate polymer derived from the chitin component of the exoskeleton of crustacea such as shrimp, crab, and crawfish. Solid wastes from the rapidly growing Lousiana crawfish processing industry are a feasible source of chitosan, together with an integrated pigment extraction process. Chitosan, with its partial positive charge, can effectively function as a polycationic coagulant in wastewater treatment (Peniston and Johnson, 1970). Earlier investigations 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 (Wu et al., 1978), and vegetable operations (Bough, 1975b), with reduction in suspended solids of 70–98%. With certain products, i.e., poultry (Bough et al., 1975) and meat wastes (Bough, 1976), reductions in the chemical oxygen demand (COD) of 60–80% have been obtained. Only a few studies have involved use of chitosan in treatment of seafood processing wastewater, mainly as a coagulant for recovery of suspended solids (Bough, 1976; Johnson and Gallanger, 1984). In addition to waste load reduction, the coagulated byproducts from food processing wastes generally contain significant amounts of protein (30–75%) and have potential applications in animal feeds (Bough and Landes, 1978).

The availability of a major concentration of chitinous wastes from the Louisiana crawfish industry, and use of derived chitosan in recovery of organic compounds of seafood origin, comprises a comparatively new area of investigation for use of this biopolymer. The current research describes utilization of crawfish chitosan for coagulation and recovery of organic compounds, notably flavor-related amino acids, from crawfish processing wastewater.

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EXPERIMENTAL SECTION

Sample Collection. Heat-processed crawfish waste, comprised of the intact cephalothorax, abdominal exoskeleton, and viscera, was collected from commercial crawfish processors, after parboiling and removal of the edible tailmeat. Material was placed into double black polyethylene bags, iced during transport, and stored at −20 °C until use.

Sources of Commercial Coagulants. Two commercial chitosans (from crab shells, practical grade), designated chitosan S and chitosan B, were obtained from Sigma Chemical Co. (St. Louis, MO) and Bioshell Inc. (Albany, OR), respectively.

Five synthetic commercial polymers were used for general comparative purposes: anionic Betz 1410, anionic Betz 1420, and cationic Betz DG-979 (Betz Laboratories, Trevose, PA); cationic Magnifloc 2535CH and cationic Magnifloc 2540C (American Cyanamid Co., Wayne, NJ). The inorganic salt used was ferric chloride ($FeCl_3 \cdot 6H_2O$, Fisher Scientific).

Preparation of Crawfish Chitosan. Chitin was prepared from crawfish shell following procedures (No, 1987) developed in our laboratory.

Preparation of chitosan was achieved by reaction of the crawfish chitin with 50% NaOH (w/w) solution at 100 °C for 30 min in air using a solid to solvent ratio of 1:10 (w/v). Immediately following deacetylation, the hot mixture was transferred to a stainless-steel beaker of ice water for rapid cooling. Chitosan was isolated by vacuum filtration, followed by washing in running tap water to neutrality, rinsing with deionized water, and drying in a forced-air oven at 60 °C for 4 h.

Preparation of Coagulant Solutions. Crawfish chitosan and commercial chitosan solutions were prepared by dissolving 10 g/L in 2% acetic acid. The five synthetic polymers and the inorganic salt were dissolved in deionized water at three concentrations: 0.5, 1, and 10 g/L.

Preparation of Crawfish Wastewater. To obtain reproducible and consistent results, crawfish process wastewater was prepared in our laboratory simulating operational procedures used in the commercial pigment extraction process (Chen and Meyers, 1982). Wastewater samples were placed in 1-gal containers and stored at −20 °C. Prior to use, the material was thawed to ambient room temperature.

Laboratory Jar Tests. The conventional jar test (Culp and Culp, 1971) was used to establish optimal conditions of pH, concentration of chitosan, and settling time. Wastewater samples (100 mL), adjusted to the desirable pH levels, and appropriate concentrations of chitosan were stirred for 2 min at 100 rpm followed by 3 min at 30 rpm. In combination studies, chitosan and an inorganic salt were added sequentially to wastewater. The treated samples were allowed to settle for 1 h, after which supernatant aliquots were withdrawn via a pipet. Turbidity was measured as nephelometric turbidity units (NTU) (Sargent-Welch S-83700 turbidimeter). In determination of optimal pH, the pH

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Table I. Characterization of Raw Crawfish Wastewater

parameter	mean ● SD[a,b]
total solids, mg/L	34813 ± 122
total dissolved solids, mg/L	29243 ± 480
total suspended solids, mg/L	5567 ± 601
fixed solids, mg/L	5543 ± 21
volatile solids, mg/L	29270 ± 135
pH	8.22 ± 0.03
chemical oxygen demand (COD), mg/L	33000 ± 218
turbidity, NTU	3500 ± 30

[a] Average of three determinations. [b] SD = standard deviation.

of the wastewater was adjusted either with 6 and 0.1 N HCl or with 6 and 0.1 N NaOH, to achieve final pH levels of 4, 5, 6, 7, and 8.

Recovery of Coagulated Solids. Optimal values of pH and chitosan concentration, based on laboratory jar tests, were applied to a 1-L sample of wastewater to recover adequate amounts of coagulated solids. These were recovered by centrifugation at 10 000 rpm for 10 min, dried in a forced-air oven for 16 h at 103 °C, and subjected to proximate and amino acid analyses.

Wastewater Analysis. Characteristics of raw crawfish wastewater samples were determined in triplicate by standard methods (APHA, 1985) for the following: total, dissolved, suspended, fixed, and volatile solids; pH; chemical oxygen demand (COD); turbidity. Values reported for turbidity, suspended solids, and COD in laboratory jar tests are averages of duplicate determinations.

Proximate Analysis. Crude protein of dry coagulated solids, calculated as N × 6.25, was determined in duplicate by a semiautomated method (AOAC, 1980). Moisture, ash, and fat were determined in duplicate by standard methods (AOAC, 1980), after which the defatted samples were used for amino acid analysis.

Amino Acid Analysis. Each 0.1 g of sample was hydrolyzed with 6 N HCl for 24 h at 110 °C under vacuum. The hydrolysates were filtered, evaporated to near-dryness, and made up to 25 mL with sodium citrate buffer of pH 2.2. A 0.2-mL aliquot was used for analysis (Beckman Model 116 amino acid analyzer).

Statistical Analysis. The data were analyzed by the analysis of variance. Means of the main effects were separated by Duncan's multiple-range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Characterization of Raw Crawfish Wastewater. Various parameters of the wasteload were determined as a base line for subsequent chemical treatment of the stream. As seen in Table I, the high level of total solids, especially dissolved solids, is particularly noteworthy. While total suspended solids can be removed from wastewater by screening, this is impractical in view of the excessive filter surface or the requirement for an excessive pressure drop across the filter. Filter clogging and replacement costs also pose problems. Thus, coagulation followed by settling is widely used for removal of suspended solids that are amenable to coagulation (Wheaton and Lawson, 1985).

High chemical oxygen demand (COD) value (33 000 mg/L) of crawfish wastewater correlates well with the equally high concentration of total dissolved solids (29 247 mg/L), as mentioned by Green and Kramer (1979). Turbidity measurement of crawfish wastewater, 3500 NTU (nephelometric turbidity unit), also can be used to measure changes in total suspended solids (TSS) and COD present in wastewater due to the high correlation between TSS, turbidity, and COD (Bough, 1975a; Karim and Sistrunk, 1985).

Optimum pH and Concentration of Crawfish Chitosan. The effect of pH on reduction of turbidity in crawfish wastewater (Figure 1) showed that lowest turbidity (597 NTU) was achieved at pH 6.0. At pH 7.0, turbidity was reduced to 610 NTU. Treatment with 150 mg/L chitosan at pH 4.0, 5.0, or 8.0 resulted in turbidity values greater than 1000 NTU.

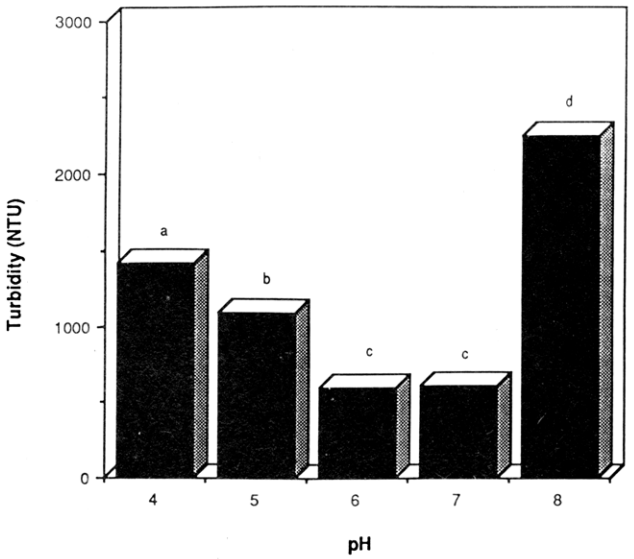

Figure 1. Effect of pH on reduction of turbidity in crawfish wastewater treated with 150 mg/L chitosan. [a-d]Means with the same letter are not significantly different at the 5% level.

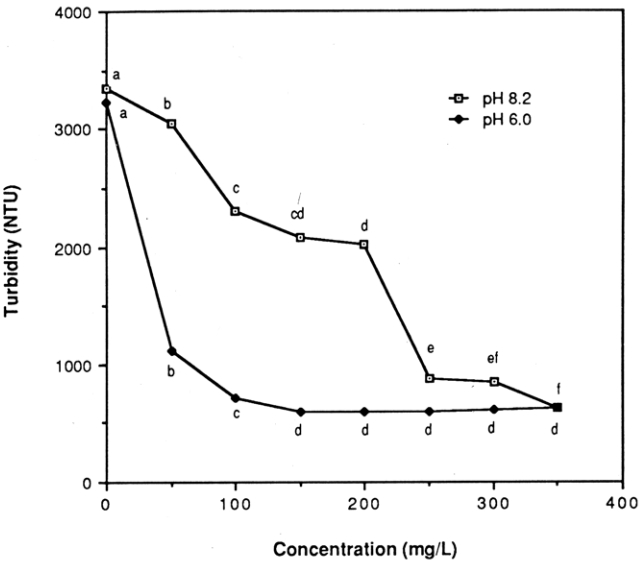

Figure 2. Changes in turbidity in response to different concentrations of chitosan at pH 8.2 and pH 6.0. [a-f]Means with the same letter among concentrations are not significantly different at the 5% level.

Changes in turbidity in response to different concentrations of chitosan at pH 8.2 (the original pH of crawfish wastewater) and at pH 6.0 (the optimum pH for treatment) are shown in Figure 2. Prior to treatment, pH of the sample was always checked. Turbidity values in the settled supernatants treated with 150 mg/L chitosan at pH 8.2 and 6.0 were 2080 and 602 NTU, respectively. At pH 6.0, chitosan treatments above 150 mg/L did not result in significant ($P > 0.05$) increases or decreases in turbidity values. In the control test (without coagulant), turbidity was reduced by 6% from 3500 to 3300 NTU at pH 8.2 and by 8% from 3500 to 3210 NTU at pH 6.0. These results demonstrate that pH adjustment is critical in determining the optimal concentration of chitosan needed to reduce turbidity.

Several workers (Bough and Landes, 1976; Moore et al., 1987) have observed restabilization of the colloidal suspension due to excessive treatment of organic polymers. However, in the present study, an increase in chitosan

Table II. Effect of Settling Time on Turbidity, Suspended Solids, and COD Values of Crawfish Wastewater Treated with 150 mg/L Chitosan at pH 6.0

sample	time, h	pH	turbidity, NTU	suspended solids, mg/L	COD, mg/L
raw	0	8.2	3500[a]	5567[a]	33 000[a]
CS[a]	0.5	–[b]	600[b]	–	–
CS	1.0	5.3	600[b]	182[b]	18 000[b]
CS	1.5	–	600[b]	–	–
CS	2.0	–	600[b]	–	–
CS	3.0	–	600[b]	–	–

[a] CS = coagulated and settled. [a,b] Means with the same letter in the columns are not significantly different at the 5% level. [b] Not determined.

Table III. Comparison of Crawfish Chitosan with Various Natural and Synthetic Polyelectrolytes Applied at a Concentration of 150 mg/L for Reduction of Turbidity in Crawfish Wastewater at pH 6.0

coagulating agent	turbidity, NTU
none	3210[a]
crawfish chitosan (+)[a]	593[b]
chitosan S (+)[b]	633[bc]
chitosan B (+)[c]	638[c]
Betz 1410 (–)	2220[d]
Betz 1420 (–)	2200[d]
Betz DG-979 (+)	688[e]
Magnifloc 2535CH (+)	2950[f]
Magnifloc 2540C (+)	2980[f]

[a] (+) and (–) indicate nature of the charge on polymer. [a–f] Means with the same letter are not significantly different at the 5% level. [b] Commercial crab chitosan from Sigma. [c] Commercial crab chitosan from Bioshell.

concentration to 350 mg/L at pH 6.0 (Figure 2) did not result in a corresponding significant increase in turbidity ($P > 0.05$). O'Melia (1972) observed that concentrated suspensions are difficult to restabilize by excessive treatment at any pH.

Effect of Settling Time. Optimal conditions of pH and concentration of chitosan from the laboratory jar tests were used to approximate optimum settling time.

Coagulation and gravity settling for 0.5–3 h gave the same reduction in turbidity by 83% from 3500 to 600 NTU (Table II). Coagulation and gravity settling for 1 h reduced suspended solids by 97% from 5567 to 182 mg/L and COD by 45% from 33 000 to 18 000 mg/L. A comparatively low reduction of COD correlates with the high level of total dissolved solids (29 246 mg/L) (Table I) in crawfish wastewater, as mentioned by Bough et al. (1975).

Comparison of Crawfish Chitosan with Commercial Chitosan and Synthetic Polymers. Crawfish chitosan was compared with two commercial chitosan products and five synthetic commercial polyelectrolytes for relative effectiveness of treatment at pH 6.0, the optimal pH of crawfish chitosan (Table III). All were applied at 150 mg/L, the most effective crawfish chitosan concentration (Figure 2).

Coagulation with crawfish chitosan resulted in a turbidity reading of 593 NTU, the lowest value observed, followed by those of chitosan S (Sigma) and chitosan B (Bioshell), i.e., 633 and 638 NTU, respectively. Changing conditions of pH and concentration for the natural and synthetic polymers would be expected to affect their relative effectiveness. In this limited study, these were used only as general reference points for the crawfish chitosan at its optimal pH. However, at 150 mg/L and pH 6.0, chitosan was equivalent or superior to the commercial polymers. These data support earlier observations (Bough, 1975a; Bough and Landes, 1976) comparing crab shell chitosan with synthetic polyelectrolytes.

Table IV. Comparison of Amino Acid Composition of Coagulated Solids from Crawfish Wastewater with Shrimp Waste Protein

amino acid[a]	content, mg/g crawfish[b]	content, mg/g shrimp[c]
aspartic acid	61.6	63.4
threonine	21.1	25.3
serine	19.1	26.7
proline	11.5	20.3
glutamic acid	121.3	91.2
glycine	17.2	25.3
alanine	43.0	31.2
valine	22.7	26.1
methionine	10.1	16.8
isoleucine	13.7	19.2
leucine	48.1	44.6
tyrosine	16.3	21.4
phenylalanine	18.8	26.9
lysine	35.5	36.4
histidine	8.1	11.2
arginine	43.5	37.2
total	511.6	523.2

[a] Tryptophan was destroyed in the acid hydrolysis. [b] Average of duplicate determinations. [c] From Toma and Meyers (1975).

Combination of Crawfish Chitosan with an Inorganic Salt. In an effort to obtain larger floc formation and greater reduction of turbidity in crawfish wastewater (adjusted to pH 6.0), chitosan was combined with $FeCl_3$ at different concentrations: i.e., chitosan, 0–200 mg/L; and $FeCl_3$, 0–1200 mg/L, respectively. However, addition of $FeCl_3$ at the various concentration did not noticeably enhance turbidity reduction. Comparable results also were observed on poultry chiller effluent by Bough et al. (1975).

Analyses of Coagulated Solids. *Proximate Analysis.* Proximate analysis of the coagulated solids recovered by coagulation with 150 mg/L chitosan at pH 6.0 showed a crude protein content of 27.1%, while average values for fat and ash were 51.7% and 3.3%, respectively. The high fat content of the coagulated solids probably resulted from the ineffective separation of the pigmented oil and water with the cream separator (Model 100, the De Laval Separator Co.) used during laboratory preparation of simulated crawfish wastewater. Although the fat layer that developed after settling was carefully removed, it is possible that the lipid in the wastewater was deposited during centrifugation. Treatment of the wastewater from the commercial pigment extraction process, in all likelihood, will result in a considerably lower fat content of the coagulated solids. The yield of dry coagulated solids was 5.97 g/L.

Amino Acid Composition. Coagulated solids used for amino acid analysis were defatted with hexane for 18 h to avoid experimental error from the high levels of fat present. The amino acid composition of the defatted coagulated solids from crawfish wastewater was analyzed and compared with that of shrimp waste protein (Table IV).

A common notable feature of the coagulated solids and shrimp waste protein is the extremely high content of glutamic acid and aspartic acid, with somewhat lower amounts of leucine, arginine, and alanine. Significantly, these five amino acids accounted for 62% of those present in the coagulated solids from crawfish wastewater and 51% of the total amino acids present in the shrimp waste protein. Few overall differences were observed in the amino acid composition of the two seafood processing byproducts.

This investigation has emphasized analysis of free amino acids in the supernatant after coagulation of suspended solids since compounds such as arginine, alanine, glutamic acid, serine, and glycine are important in crustacean sensory attributes (Hayashi et al., 1981; Konosu and Yama-

guchi, 1982). Other flavor-related compounds, i.e., a variety of nucleotides (Meyers and Sonu, 1974), have been found in shrimp processing discharge streams. Further studies are needed to characterize the total organic compounds present in the crawfish wastewater for their potential usage in seafood products.

This investigation has demonstrated that crawfish chitosan is an excellent coagulant for recovery of organic compounds from crawfish processing wastewater. The recovery of coagulated solids and their use as feed additives is of particular interest in view of its relevance to organically rich seafood processing wastes in general. Absence of large levels of inorganics, especially iron and aluminum, in the chitosan-separated solids may be beneficial in view of the potential usage of the chitosan as a feed additive. Application of chitosan to recover organic compounds or byproducts from seafood discharge streams should involve total integration of discharge and byproduct generation processes. However, reduction of effluent BOD, apart from the value of the recovered product, is in itself a worthwhile objective. The economics of recovery of organic compounds from seafood processing operations must be examined, since conditions vary from plant to plant as does composition of the organic components discharged.

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Registry No. Chitosan, 9012-76-4; aspartic acid, 56-84-8; threonine, 72-19-5; serine, 56-45-1; proline, 147-85-3; glutamic acid, 56-86-0; glycine, 56-40-6; alanine, 56-41-7; valine, 72-18-4; methionine, 63-68-3; isoleucine, 73-32-5; leucine, 61-90-5; tyrosine, 60-18-4; phenylalanine, 63-91-2; lysine, 56-87-1; histidine, 71-00-1; arginine, 74-79-3; Betz 1410, 119365-96-7; Betz 1420, 119365-97-8; Betz DG-979, 119365-98-9; Magniflock 2535CH, 100843-10-5; Magniflock 2540C, 100843-09-2; Chitosan S; Chitosan B.

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