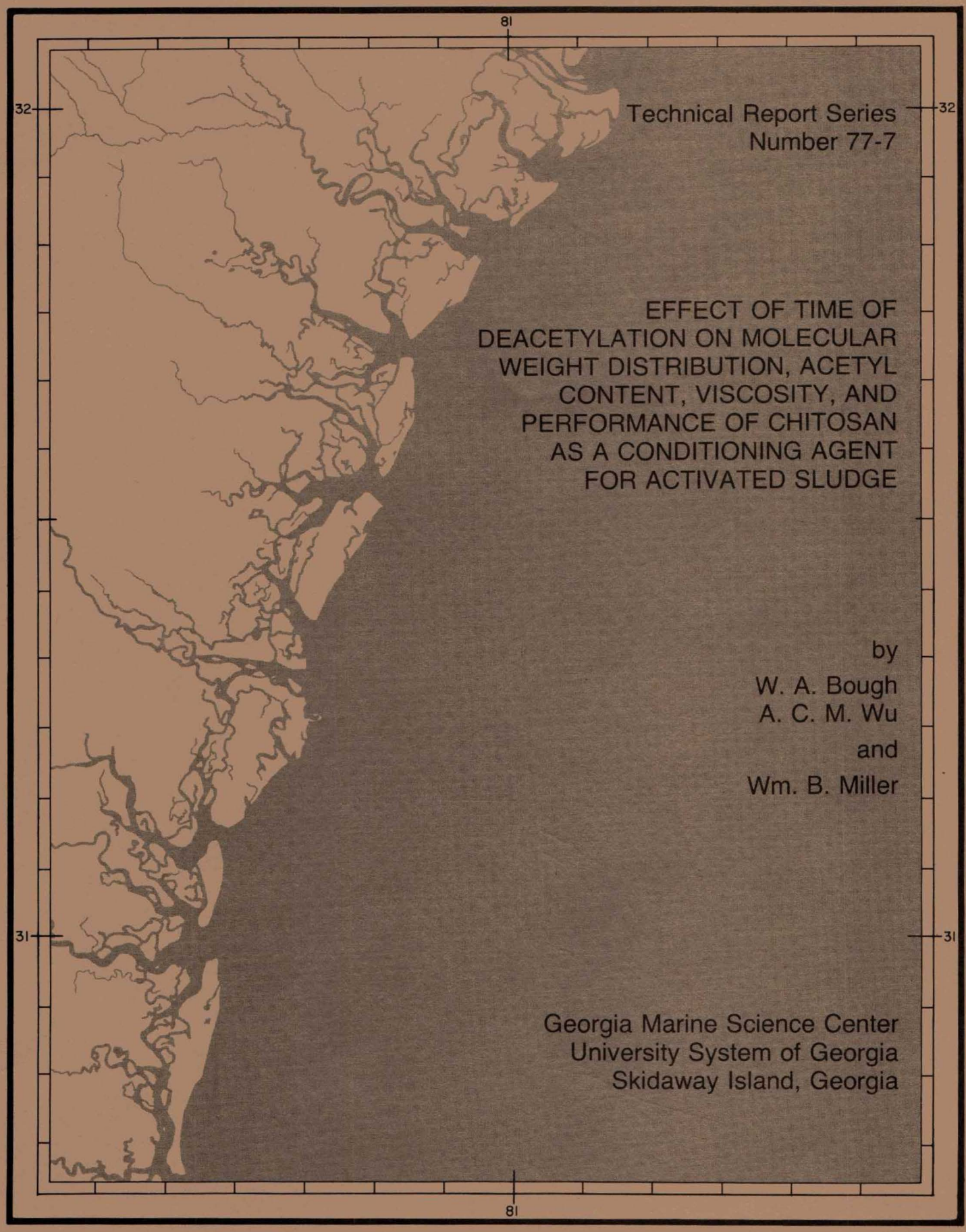

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EFFECT OF TIME OF DEACETYLATION ON MOLECULAR WEIGHT DISTRIBUTION, ACETYL CONTENT, VISCOSITY, AND PERFORMANCE OF CHITOSAN AS A CONDITIONING AGENT FOR ACTIVATED SLUDGE

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Chitosan Manufacturing Variables

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Dried shrimp hulls were ground to 1 mm, demineralized with 0.5 N HCl, deproteinized with 1% NaOH, and then deacetylated with 50% (w/w) NaOH at 100°C for 0.5, 1, 2, 3, 4, and 5 hours. Analyses of these chitosan products differing only in time of deacetylation showed that ash and moisture contents ranged from 0.05 to 0.2 and 5.6 to 10.0, respectively. Degree of deacetylation decreased sharply in the first 30 minutes from 14% to 67%, then slowly decreased to 79% in 5 hours. Viscosity also decreased sharply in the first one hour, then in the next 2 to 5 hours slowly decreased to about 200 cps at 10 g/l in 2% acetic acid solution. Similar trends were obtained for molecular weight distribution data as determined by high performance liquid chromatography. Weight average molecular weights values decreased from 1.487 million at 0.5 hour deacetylation time to 0.667 million at 5 hours. The corresponding number average molecular weight values were 0.322 and 0.129 million, respectively. The effectiveness of the different chitosan products as coagulants in dewatering activated sludge grown on vegetable processing wastes was highest for the sample deacetylated for 2 hours. This product was more effective than those deacetylated for shorter or longer times than two hours. Correlation of effectiveness vs. viscosity or molecular weights of products was attempted for products deacetylated for 0.5, 1, and 2 hours. Molecular weight distribution data showed better correlation with effectiveness as a waste treatment agent than did viscosity data.

INTRODUCTION

Chitosan is a high molecular weight (MW) cationic polymer derived from chitin, a polymer of N-acetylglucosamine residues. The monomer units are linked by beta-1,4-glycosidic bonds. Chitin can be isolated from the exoskeleton of crustaceans as the insoluble residue remaining after extraction of proteinaceous matter and mineral salts. Several methods for the isolation of chitin and for the conversion of chitin to chitosan will be reviewed here.

Three basic steps in the manufacture of chitosan from shrimp and crab wastes are: demineralization and deproteinization of raw material to produce chitin, followed by deacetylation of chitin to give chitosan. Conditions of time, temperature, choice of reagents, concentrations, and raw waste materials are all variants in the process of manufacturing chitosan. Variation of methods produces chitosan products which may differ in terms of solution viscosity, extent of deacetylation, polymer degradation, and general effectiveness of application testing.

Most methods for obtaining chitin from crustacean wastes include treatment with dilute acid at ambient temperature to remove inorganic salts (primarily calcium carbonate), and treatment with dilute alkali at elevated temperatures to solubilize extraneous protein. Some such methods are those of Rigby (1936a), Stacey, Hackman, and Broussignac (Muzzarelli, 1973a). Various acids and alkalies are effective, as are various concentrations, but most commonly used are dilute hydrochloric

acid (1-5%) and dilute sodium hydroxide (1-10%). Notable exceptions are the methods of Whistler and BeMiller where demineralization is accomplished in 37% HCl at -20°C, and the method of Roseman et al. where demineralization is effected in 90% formic acid at ambient temperature (Muzzarelli, 1973a). The durations of acid and alkali treatments and the temperatures at which they are conducted may differ widely and likewise cause degradation of the chitin superstruction to varying degrees. Durations range from less than one hour to several days; temperatures range from less than 0°C to 100°C. With certain methods, the chitin is even demineralized and/or deproteinized twice (Rigby, 1936a; Muzzarelli, 1973a).

Some of these methods include treatments which are too harsh if it is desired that a product be pure chitin, substantially free from protein and minerals, but in a native or highly undegraded form where deacetylation has been avoided. Certain methods, such as those of Foster and Hackman, Takeda and Katsuura, and Broussignac have been developed to minimize degradation by the use of milder conditions. These methods accomplish demineralization and deproteinization by employing such agents as enzymes and EDTA at controlled pH and at moderate temperatures (Muzzarelli, 1973a). The result is reportedly a purer form of chitin whose tertiary structure and molecular size may be closer to those of chitin in its native form.

The conversion of chitin (poly-N-acetylglucosamine) to chitosan is achieved by the removal of some or all of the acetyl groups from the polymer, liberating free amine groups. This deacetylation step involves the use of concentrated alkali at elevated temperatures with the duration of treatment varying with the type of product desired. According to Rigby (1936a), six factors affect the extent of deacetylation: concentration of the caustic alkali, temperature of deacetylation, time of reaction, previous treatment of the chitin, particle size, and density of the chitin. The latter two factors affect the penetration rate of the alkali. For the alkaline hydrolysis reaction, conditions are sought which in a reasonable time will sufficiently deacetylate the chitin to yield a chitosan product which is soluble in dilute acetic acid but which is not significantly degraded.

Horwitz, for example, fused chitin with solid potassium hydroxide at 180°C, and in only 30 minutes he obtained a product which was 95% deacetylated. However, when tested by dialysis for degradation, his "chitosan" polymer was found to have a chain length of only about 20 units. Obviously this chitosan had been highly degraded, since values for the molecular weight of chitosan have been reported as 120,000 and greater (Muzzarelli, 1973a; Peniston and Johnson, 1970). Rigby warned, "When highly concentrated caustic alkali solutions are used, the treatment must be conducted at a lower temperature, since concentrated caustic alkali at elevated temperature causes drastic changes on the chitin molecule, as evidenced by ammonia evolution, water solubility of the product, and the crystalline nature of the salts of the product." Heating at 160°C in 50% potassium hydroxide also produced a degraded chitosan product.

On the other hand, though care must be taken to avoid degrading the polymer, if conditions are too mild, the resulting product will not be soluble in weak acid. Rigby reported that at a concentration of 5% sodium hydroxide at 150°C, a deacetylation time of 24 hours was required to give a soluble product. At 100°C in 40% NaOH, 18 hours were needed, and with 50% NaOH at 100°C, only 1 hour was required to achieve the same result (Rigby, 1936a). Rigby also used other manufacturing conditions, including: (a) 4 hours at 110°C in 40% NaOH (Rigby, 1936b); (b) 6 hours at 115°C in 40% NaOH also referred to as the method of Rigby and Wolform (Muzzarelli, 1973b); (c) 1 hour at 100°C in 50% NaOH; and (d) 48 hours at 100°C in 50% NaOH. Reportedly, 6 hours at 115°C in 40% NaOH gave a chitosan product which was 82% deacetylated, while 48 hours at 100°C in 50% NaOH "approached complete deacetylation" (Rigby, 1936a).

While such variations exist with respect to time and temperature of deacetylation and concentration of alkali, certain factors are common to most methods: sodium or potassium hydroxide is typically used in a concentration of 40% or more on a weight-weight basis, and elevated temperatures of 100°C or higher are generally used. With a given method and a given set of conditions, increased deacetylation time decreases the viscosity of a solution of the resulting chitosan product and finally, "free access of oxygen to the chitin during the deacetylation step has a substantial degrading effect..." (Rigby, 1936a). Thus, most methods require that an inert atmosphere be supplied or at least that exposure to air and oxidation be limited. Such oxidation can be simulated by the use of some chemical agent, such as hydrogen peroxide, with the result that the viscosity of a solution of an oxidized sample is lower than that of an identical but undegraded sample (Rigby, 1936a).

Uses of chitosan are more varied than the methods for producing it, with most being related to its macromolecular polycationic nature. Rigby studied the film-forming characteristics and adhesive qualities of chitosan solutions. He noted that the solutions of highest viscosity (from several hundred to thousands of poises) have the best film-forming capabilities, producing films of greater tensile strength (Rigby, 1936b). In his work with chitosan adhesives, he showed that by thorough drying, heating, or special chemical treatment, these glues (and also chitosan films) could be made water insoluble.

Peniston and Johnson (1970) have studied the use of chitosan as a coagulating agent to reduce the turbidity of clay suspensions. In one experiment using a suspension of kaolinite clay, a higher viscosity, less-deacetylated chitosan sample was more effective (reduced the turbidity of the suspension to a greater degree) than did a less viscous more-deacetylated sample. However, using a different suspension, such as montmorilonite clay, these results were reversed, i.e., the less viscous more deacetylated sample gave the greatest turbidity reduction.

Chitosan's potential as a coagulating agent for suspended solids in the treatment of food processing wastewater and activated sludge

suspensions has been shown in earlier reports from this laboratory (Bough, 1975; Bough, 1976). The purpose of this study is to produce chitosan samples from a single batch of shrimp hulls which have received identical treatment except for deacetylation time. We propose to manufacture samples whose only differences will be due to systematic variation of deacetylation time resulting in differences in their percentages of deacetylation, molecular weights, and viscosities in solution. Measurements will be made of each of these parameters, and an attempt will be made to correlate one or more of them to the efficiency of chitosan as a coagulating agent for dewatering of activated sludge (Bough et al., 1976).

EXPERIMENTAL

Materials. Shrimp hulls were collected from a processing plant, iced during transport, and dried the next day in a forced-air oven at 103°C for 24 hours. Dried shrimp hulls were ground in a Wiley mill to pass a 1 mm screen prior to any chemical treatment. A total of 3600 g of this shrimp meal was divided into four portions of 900 g for ease of processing in the laboratory.

Activated sludge was obtained from a biological treatment plant operated by a local commercial vegetable processing plant. It was collected daily from the underflow of the clarifier being recycled back to the biological treatment basins. The turbidity of the sludge was adjusted to a reading of 1250 Formazine Turbidity Units, which resulted in a suspended solids concentration of approximately 7000 mg/l, before use. The pH of the sludge samples remained at 7.0 throughout the study. Suspended solids in the daily sludge samples were routinely determined and these values used in the calculation of specific resistance as described in Section J.

Methods

A. Demineralization. Each portion (900 g) of dry shrimp hulls was treated with 10 l of 0.5 N HCl for 30 minutes at room temperature with constant stirring. This represented a 10% excess of HCl over the stoichiometric amount of ash considered as calcium carbonate in the dry shrimp hulls. The demineralized material was then collected on 60-mesh and 200-mesh screens and washed to neutrality with deionized water.

B. Deproteinization. The wet residue from above was treated with 9 l of 1% (w/w) NaOH for one hour at 65°C with constant stirring. The wet chitin was collected on 60-mesh and 200-mesh screens, washed to neutrality with deionized water, and then dried overnight at 85°C in a forced-air oven. The ash contents of the individual batches of chitin were 0.83, 0.54, 0.58, and 0.47% on a dry weight basis. The four batches of chitin were mixed thoroughly before proceeding with deacetylation.

C. Deacetylation. Deacetylation of chitin to produce chitosan was performed in a 4 liter resin reaction kettle. Four holes in the kettle top provided mounts for a thermometer, gas delivery tube, overhead stirrer, and a rubber stopper to plug the sampling port. The kettle was heated with a 700 watt heating mantle connected to a variable transformer.

The apparatus was assembled and the kettle was charged with 400 g of 50% (w/w) NaOH. Temperature was brought to 100°C, and the stirrer and nitrogen gas were turned on. The clearance between the stirrer shaft and the rubber stopper through which it extended served as an exit for the nitrogen purge. A gas flow of about 0.5 liters per minute was maintained. When the temperature, gas flow, and stirring rate were constant and adjusted properly, 200 g of dry chitin was added, making the ratio of dry NaOH to chitin 10:1 (w/w). Readjustment of the stirrer sometimes was necessary to aid in bringing the chitin into suspension. When chitin addition was complete, the rubber stopper was replaced and the timer started. Total volume of the reaction mixture was about 3 liters. The temperature, gas flow, and stirring rate were monitered and adjustments made as necessary to maintain constant conditions.

At specified times, samples were taken from the reaction mixture. Samples were drawn with vacuum through 1/4 inch Teflon tubing into an appropriate size vacuum flask. Deionized water and ice were added to this flask prior to drawing the sample, effectively lowering the reaction temperature as the sample entered the flask.

The chitosan samples were collected on 60-mesh and 200-mesh screens, washed to neutrality with deionized water, and dried overnight (18 to 20 hours) at 70°C in a forced-air oven. It was found to be of benefit to allow the dried product to cool in free exposure to atmospheric moisture for several hours before being transferred to a storage container. In this way, the chitosan absorbed atmospheric moisture before a determination was made of its moisture content. Thus samples could be stored with little subsequent change in moisture contents.

D. Moisture and Ash. Values for percent moisture and percent ash are necessary so that solutions of chitosan can be prepared on the basis of weight of chitosan per unit volume and that determinations for percent free amine and percent acetyl be expressed on a moisture- and ash-free basis.

Moisture content was determined in duplicate, in standard covered aluminum moisture pans, on 0.5 g to 1.0 g samples (weighed to the nearest 0.1 mg) by heating at 95°C *in vacuo* for five hours (Horwitz, 1970).

Products were analyzed for ash content in duplicate in porcelain crucibles on 1 gram samples (weighed to the nearest 0.1 mg) by heating at 600°C in a muffle furnace for two hours (Horwitz, 1970).

E. Viscosity. Viscosity was determined on 500 ml solutions of chitosan in 2% acetic acid which had been prepared to contain 10 g/l chitosan on a moisture- and ash-free basis. A Brookfield Model RVT spindle-type viscometer was used for the measurements which were made at 20°C with spindle #1 at speeds of 2.5 rpm and 5 rpm.

After viscosities had been determined in the 2% acetic acid solvent, sufficient sodium acetate was added to each of the solutions to bring the salt concentration to 0.1 M, and the viscosity measurements were repeated. This was done to observe the effect of the added salt on viscosity.

F. Free Amine Content. Determination of percent free amine (w/w) was done in duplicate by the method of Broussignac (1968) on 0.5 g samples (weighed to the nearest 0.1 mg). With this method, the free amine groups were found beginning to titrate at pH 4; lower than this pH, deacetylated amine groups were assumed to be 100% positively charged. The end point of the titration was around pH 7.8; thus, the apparent peak of amine groups on chitosan was about pH 5.9. A test was made to discover whether the obtained value for percent free amine would change if the sample was subjected to the conditions of drying necessary for the determination of moisture content. No such change was observed. Performing a moisture determination on a sample and then performing a free amine determination on the same sample made possible a more accurate correction for moisture content and a saving on product.

G. Acetyl Content. Determination of percent acetyl (w/w) was made according to the method of Lemieux and Purves (1947). A comparison was made of values calculated for percent deacetylation from the free amine assay and those values calculated from the acetyl assay.

H. Percent Deacetylation. Percent deacetylation is another way of expressing the degree of acetylation. It is the ratio, expressed in percent, of total number of deacetylated glucosamine units to total number of chitosan monomer units. This parameter enables us to convert either percent acetyl or percent free amine into the same units, so that results obtained from the two predescribed methods can be compared. Percent deacetylation could be calculated directly from either percent acetyl or free amine data as follows:

$$\% \text{ Deacetylation} = 203.2 \times \frac{\% \text{ Free Amine}}{(16.02 + 0.4204 \times \% \text{ Free Amine})}$$

or

$$\% \text{ Deacetylation} = \frac{(4305 - 203.3 \times \% \text{ Acetyl})}{(43.05 - 0.4204 \times \% \text{ Acetyl})}$$

It shows that 0% free amine or 21.19% acetyl are equivalent to 0% deacetylation, and 9.94% free amine or 0% acetyl are equivalent to 100% deacetylation.

I. Molecular Weight Determination. Molecular weight was determined with the use of high performance liquid chromatography (HPLC) according to the method of Wu et al. (1976). Data were based on duplicate runs on three replicate batches of chitosan products from each deacetylation time tested. Samples were prepared as 5 g/l in 0.33 M acetic acid/0.1 M sodium acetate solvent (pH 4.15). Injection size was 100 microliter for all samples. Flow rate was set at 1 ml/min. Temperature was normal ambient. The column configuration used was:

Column	Pore Size of Packing Material (Angstroms)
1 ft. x 1/4" O.D.	2500
1 ft. x 1/4" O.D.	1500
6 ft. x 1/8" O.D.	550
2 ft. x 1/8" O.D.	250
2 ft. x 1/8" O.D.	100
2 ft. x 1/8" O.D.	40

Dextran standards were used for calibration of MW. The linear region of the standard curve was $\text{Ln } M_i = 24.8041 - 0.8520 \times V_e$ where M_i was MW of i^{th} species eluted at Ve (elution volume in ml).

J. Product Efficiency as a Coagulant. Efficiency of different chitosan products dissolved in 2% acetic acid was measured by determining the specific resistance (Culp and Culp, 1971) of activated sludge suspensions which had been treated with the chitosan product at a prescribed dosage of 2 to 20 mg/l (w/v) final concentration. A composite sample was prepared for each separate deacetylation time tested by combining three replicate products (Batches II - IV) made at each time. Each composite sample was tested at six different concentrations and repeated three times on batches of sludge collected and tested on three consecutive days. Whatman No. 4 filter paper was used in an 11 cm Buchner funnel, which was attached via a vacuum adapter to a 500 ml cylindrical graduated separatory funnel. A 500 ml sample of sludge was poured into the Buchner funnel after the vacuum was started, and the volume of filtrate collecting in the vessel was recorded at five second intervals, beginning at 15 seconds and stopping at 45 seconds.

The slope of the time/filtrate volume vs. filtrate volume line was determined by linear regression analysis of the data and used in the following equation to determine the specific resistance (Culp and Culp, 1971):

$$r = \frac{2bPA^2}{\mu c}$$

Where b = slope (sec/cm^6)

P = filtration pressure (g/cm^2)

A = area, cm^2

μ = filtrate viscosity (poises)

c = suspended solids (g/ml)

r = specific resistance (sec^2/g)

The best fitting quadratic equation was then developed to describe the relationship between specific resistance values (r) at six different chitosan concentrations (X). This fitted equation was obtained using a Tektronix desktop computer (Tektronix Inc., Beaverton, Ore.). By letting the first derivative of the equation be zero, the X and r values at the inflection point of the equation could be determined. The product of the two values, Xr, was termed optimum equivalent dosage (OED). The OED value was used because it is a single value for comparing different products at their optimum concentrations (X) corresponding to maximum effectiveness at the inflection point of the curve where dr/dx = 0. The lower the OED value, the more effective the product. These values were compared by the Duncan's Multiple Range Test to determine whether differences in product performance were statistically significant and ranked according to their magnitude.

RESULTS AND DISCUSSION

Characterization of Chitosan Products. Tables 1 and 2 summarize the results obtained on four replications of the manufacturing procedure employed in this study for chitosan. Results of analyses for moisture, ash, viscosity, free amine and acetyl content are shown. The procedure for Batch I differed from Batches II - IV in that Batch I was brought up to temperature with chitin in the presence of the hydroxide. Therefore, this batch received a longer deacetylation treatment and was kept separate. It was used only for the purpose of comparing the free amine and acetyl assay methods (Table 1). Similar results on percent deacetylation were obtained with both methods.

The moisture in samples of Batches II - IV (Table 2) were all below 10%, ash contents ranged from 0 to 0.25%. Viscosity of the samples varied with the time of deacetylation as expected. Slightly different results were also obtained, depending upon whether samples

TABLE 1

COMPARISON OF FREE AMINE AND ACETYL ASSAYS IN MEASURING THE DEGREE OF DEACETYLATION OF CHITOSAN PRODUCTS (PRODUCED BY VARIOUS TIMES OF DEACETYLATION)

Sample	Percent Free Amine Content			Percent Deacetylation	Percent Acetyl Content			Percent Deacetylation
	Run 1	Run 2	Average		Run 1	Run 2	Average	
I-A	7.73	7.64	7.68	81.11	4.49	4.38	4.43	82.64
I-B	7.92	7.79	7.85	82.60	4.22	3.90	4.06	84.17
I-C	7.95	7.96	7.95	83.47	3.29	3.59	3.44	86.67
I-D	8.30	8.41	8.35	86.92	3.32	3.49	3.40	86.81
I-E	8.17	8.14	8.15	85.20	3.00	2.69	2.84	89.04

TABLE 2

FREE AMINE CONTENT AND CALCULATED PERCENT DEACETYLATION OF VARIOUS CHITOSAN PRODUCTS PRODUCED BY DIFFERENT TIMES OF DEACETYLATION

Sample	Time of Deacetylation	% Moisture	% Ash	% Free Amine			% Deacetylation
				Run 1	Run 2	Avg.	
II-A	0.5 Hour	5.62	0.085	5.76	6.12	5.94	65.17
-B	1.0	6.45	0.046	6.68	6.66	6.67	72.00
-C	2.0	6.29	0.213	7.16	7.27	7.21	76.94
-D	3.0	6.53	0.244	7.25	7.23	7.24	77.17
-E	4.0	7.34	0.138	7.40	7.54	7.47	79.22
III-A	0.5	9.46	0.098	6.38	6.28	6.33	68.85
-B	1.0	10.01	0.119	6.86	7.04	6.95	74.55
-C	2.0	9.06	0.119	7.09	7.14	7.11	76.05
-D	3.0	8.57	0.168	6.98	6.98	6.98	74.82
-E	4.0	8.31	0.030	7.32	7.33	7.32	77.93
-F	5.0	9.26	0.000	7.57	7.42	7.49	79.44
IV-A	0.5	8.25	0.030	6.28	6.25	6.26	68.24
-B	1.0	8.12	0.040	6.65	6.58	6.61	71.50
-C	2.0	7.72	0.079	6.96	6.99	6.97	74.78
-D	3.0	6.60	0.070	7.21	7.09	7.15	76.36
-E	4.0	7.62	0.098	7.41	7.36	7.38	78.46
-F	5.0	9.49	0.143	7.45	7.38	7.41	78.73
Chitin[2]	0	3.55	0.532	--	--	--	13.66[1]

[1]Percent deacetylation calculated from percent acetyl determined by the method of Lemieux and Purves.

[2]Same batch of chitin used for the deacetylation study.

were dissolved in only 2% acetic acid. The three different curves in Figure 1 are for Batches II, III and IV. Had Batch I been graphed, its curve would have fallen below all the others, because of the longer time of deacetylation due to the warming-up period. The values shown in Figure 1 corresponding to 0.5 hour of deacetylation show the greatest deviation among replications. Even at 1.0 hour, the viscosities of the products were still scattered. The deviation may be due to the presence of undissolved particles. However, for determinations on samples deacetylated for two hours and longer, viscosity was quite reproducible. Figure 2 shows the viscosity of the same samples dissolved in 2% acetic acid containing 0.2 M sodium acetate. The presence of salt decreased the viscosities of most samples, due to minimizing the interaction among chitosan molecules, as previously reported by Lee (1974). The shapes of these curves were basically similar to those in the 2% acetic acid solvent. The influence on viscosity due to the presence of sodium acetate was greater on samples with higher viscosities than on those with lower viscosities.

For determining the degree of deacetylation, the wet chemical method of Lemieux and Purves (1947) was compared to Broussignac's method (1968) of direct titration for free amino groups. Figure 3 shows results obtained on Batch I samples analyzed in duplicate by the acetyl assay shown on the dotted line and in triplicate for the free amine assay shown on the solid line. These results are expressed in terms of percent deacetylation in order to compare the two methods. Results in Figure 3 show that satisfactory agreement was obtained to justify use of the free amine titration in place of the longer and more tedious wet chemical method. Figure 4 shows the application of this method to Batches II - IV and presents the average of two measurements at each particular time. For example, the results obtained for II-A, III-A and IV-A in Table 2 were averaged and plotted at 0.5 hour, and the range of values is indicated by the three points at 0.5 hour. The point shown at zero time is the percent deacetylation of the chitin prepared in this study, as determined by the wet chemical method, because chitin was not soluble and thus not titrable. These results show that deacetylation for 0.5 hour resulted in a product being 62% deacetylated, compared to 13.5% for the starting material, chitin. Increasing periods of hydrolysis in the presence of 50% (w/w) NaOH at 100°C, as employed in this study, increased the percent of deacetylation to 75.9% at 2 hours and 79.1% at 5 hours. These results will be discussed further in relation to the MW distribution of these samples.

Table 3 shows the results obtained on Batches II - IV for the weight average and number average molecular weights ($\overline{M}_w$ and $\overline{M}_n$). The mean values and standard deviations obtained from the three replications at a particular deacetylation time are shown. For example, the mean MW obtained for products deacetylated for 0.5 hour (products II-A, III-A and IV-A) was 1,487,000 for the $\overline{M}_w$ and 322,000 for the $\overline{M}_n$. The polydispersity ($\overline{M}_w/\overline{M}_n$) and the values obtained for molecular weights corresponding to chromatogram peaks are also shown in Table 3. The mean values for $\overline{M}_w$, peak MW, and $\overline{M}_n$ are shown in Figure 5. The dashed lines

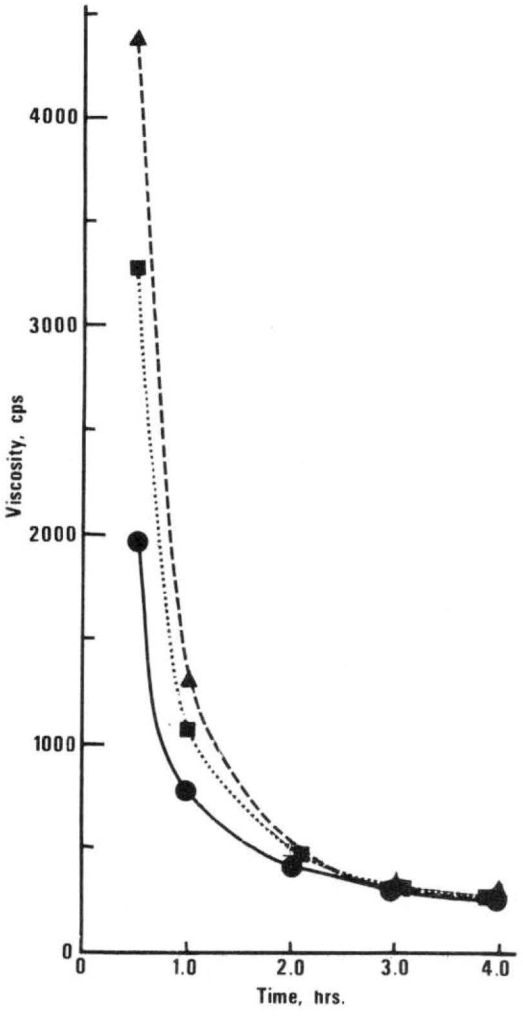

FIGURE 1. Viscosities of different chitosan products dissolved in 2% (w/v) acetic acid at concentrations of 10 g/l (w/v). The time refers to the time of deacetylation at 100°C. Three replications are shown as three curves; Batch II (circles), Batch III (triangles), and Batch IV (squares), being measured at viscometer speeds of 5, 2.5, and 2.5 rpm, respectively, with the No. 1 spindle at 20°C.

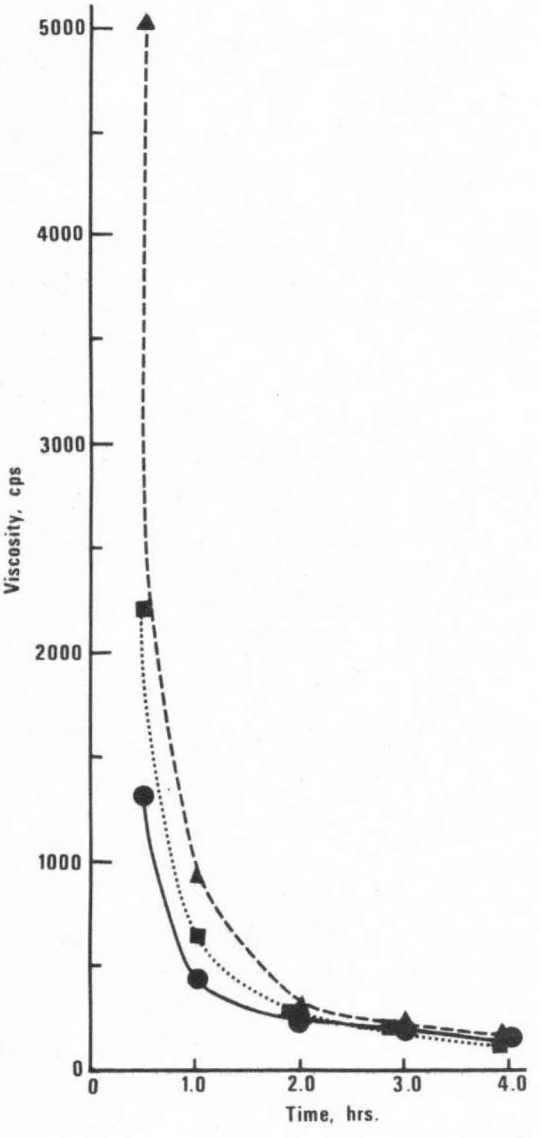

FIGURE 2. Viscosities of different chitosan products dissolved in 2% (w/v) acetic acid with the addition of 0.1 M sodium acetate. Other conditions are the same as those described in Figure 1.

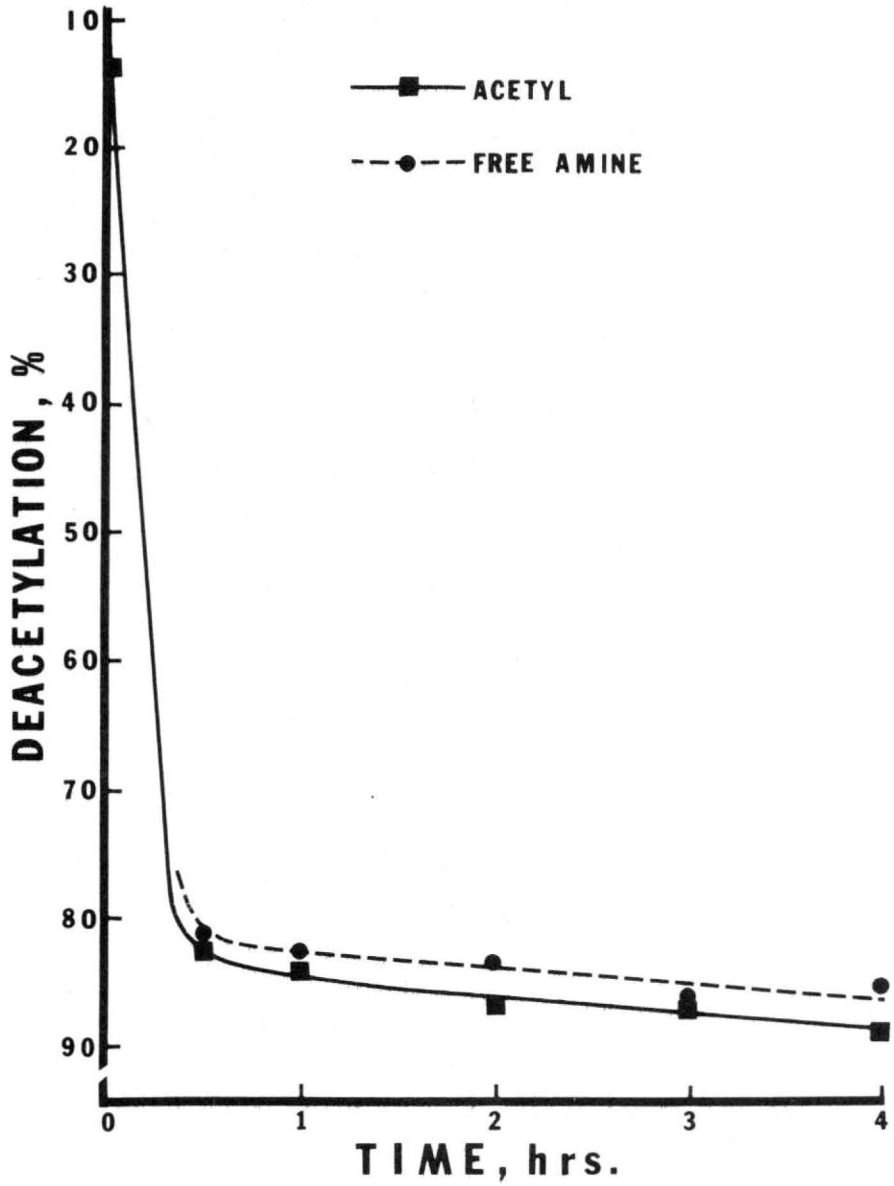

FIGURE 3. Percent deacetylations of Batch I chitosan products produced at different times of deacetylation. Two methods of determination for acetyl contents were compared: (a) free-amine method (circles) and (b) acetyl method (squares).

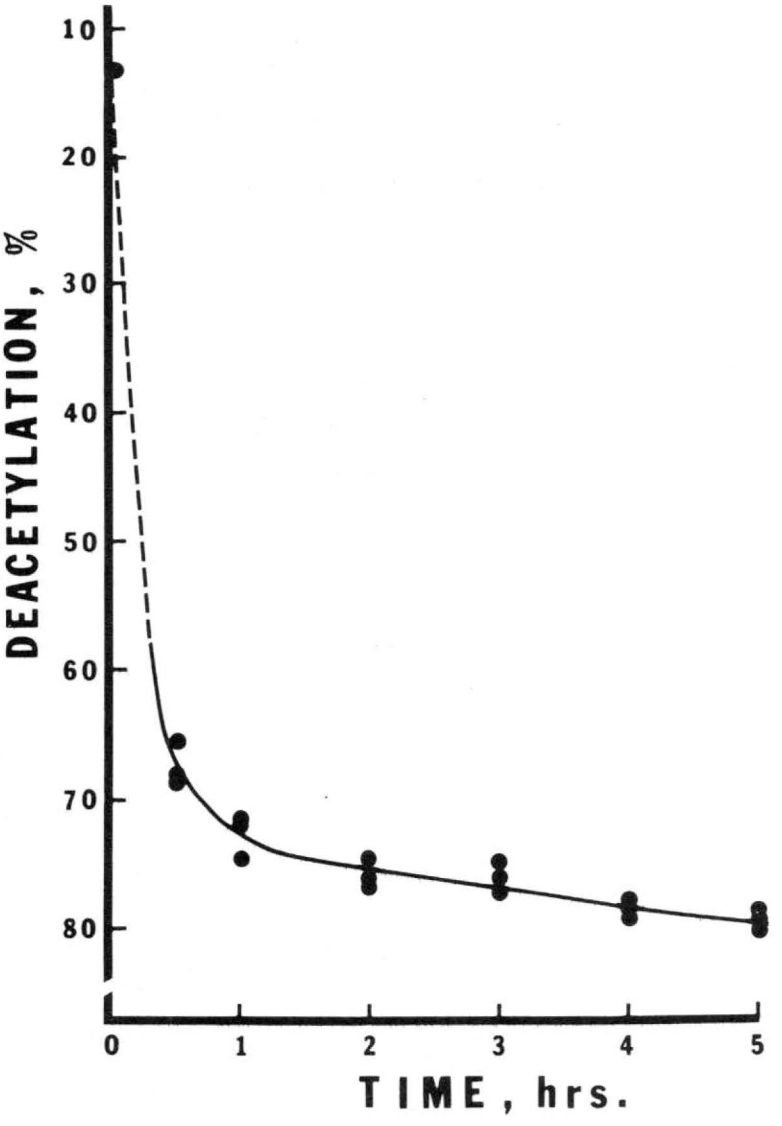

FIGURE 4. Percent deacetylations of Batches II, III, IV chitosan products produced at different times of deacetylation. The free-amine method was used for all samples except for chitin at zero time which was measured by the acetyl method.

TABLE 3

MOLECULAR WEIGHT DISTRIBUTIONS[3] OF CHITOSAN SAMPLES PREPARED BY DIFFERENT DEACETYLATION TIMES DISSOLVED IN ACETIC ACID-SODIUM ACETATE (0.33 M-0.1 M) AND DISTILLED WATER AT pH 4.15

Sample	Time (Hours)	$\overline{M}_w \times 10^{-3}$	$\overline{M}_n \times 10^{-3}$	D[1]	Peak MW $\times 10^{-3}$
A	0.5	1487 ± 98	322 ± 27	4.63 ± 0.18	1413 ± 141
B	1.0	1142 ± 94	232 ± 21	4.93 ± 0.25	1081 ± 111
C	2.0	925 ± 63	186 ± 8	4.98 ± 0.19	910 ± 38
D	3.0	846 ± 24	177 ± 7	4.79 ± 0.15	864 ± 61
E	4.0	775 ± 61	161 ± 12	4.81 ± 0.09	773 ± 42
F	5.0	667 ± 26	139 ± 8	4.80 ± 0.16	625 ± 31
4-74[2]	--	682 ± 22	129 ± 3	5.28 ± 0.2	463 ± 0

[1]D is dispersity ($\overline{M}_w/\overline{M}_n$).

[2]Sample 4-74 is a commercial product prepared by a patented method (Peniston and Johnson, 1970).

[3]Averages and standard deviations of two replicant analyses on chitosan products produced in Batches II, III, and IV.

for $\overline{M}_w$ and peak MW clearly show that increasing periods of hydrolysis had its greatest effect upon these parameters.

Statistical comparison between the MW distributions (Figure 5) and viscosities (Figures 1 and 2) of the chitosan products produced in this study showed that there were fair linear correlations between them. The linear correlation of Ln $\overline{M}_w$ and Ln viscosity measured in the solvent with 0.1 M sodium acetate gave the best correlation coefficient, which was 0.98. This relationship is described by the Staudinger equation (Log[n] = log k + a Log $\overline{M}_w$). Based on the above analyses, it appears that viscosities measured in a suitable solvent can predict the sizes of chitosan products, particularly when products are only different in molecular sizes and have minimum differences in other aspects such as manufacturing variables and raw material sources. Correlations between $\overline{M}_w$ (or $\overline{M}_n$) and percent deacetylation, were both about -0.92 which suggested that during the deacetylation, both decrease in molecular size and increase in percent deacetylation occurred in a similar manner.

Waste Treatment Effectiveness. Initial experiments with methods to evaluate the different polymers suggested that the specific resistance test, where filtrate volumes are measured at several time intervals, would be a more accurate method than the Buchner Funnel test employed previously. In a previous study (Bough et al., 1977b), polymers were evaluated using the Buchner Funnel filtration test, which basically compared the volume of filtrate collected at 30 seconds. These measurements were made with six different concentrations of chitosan and repeated six times for each concentration and each different product. The results were analyzed by regression analysis and fitted into equations, and coefficients of determination (R^2) were calculated. Table 4 shows these coefficients developed for an intercept equation, which accounted for differences in intercepts obtained on different replications and the coefficients for a generalized regression equation (Bough et al., 1977b). For example, in the case of Product III-A, the R^2 value for the intercept equation was 89.1% (viz. 89.1% of the data points were explained by this equation) and that for the generalized equation was 52.9%, when the Buchner Funnel test was employed. In the present study, the specific resistance test was sufficiently accurate to account for an R^2 value of 97.3% for the intercept equation and 96.6% for the generalized equation. Becuase of the small difference between the accuracy of the intercept equation and the generalized equation and the simplicity of the latter, we elected to analyze the following waste treatment data by the generalized equation corresponding to the response curve of specific resistance vs. chitosan dosage (mg/l).

Figure 6 shows a comparison of the different products compared at their optimum dosage. This optimum was taken as the point of inflection calculated for the response curve of specific resistance (r) vs. chitosan concentration (X). These values were calculated for each product. Specific resistance decreased as the chitosan concentration increased until the inflection point was reached, and the shape of the resulting curves approximated that of a 2nd degree polynomial equation. The data

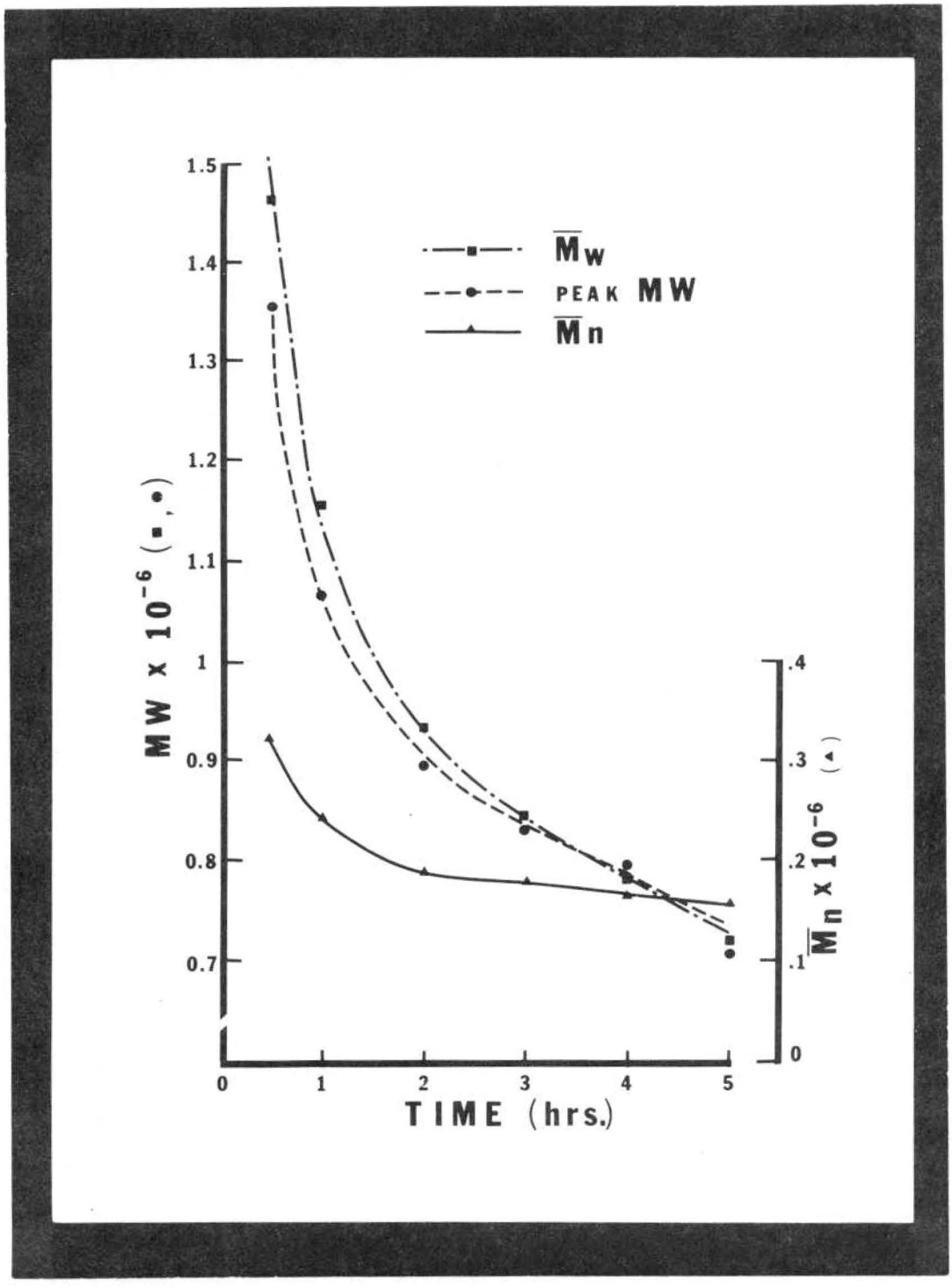

FIGURE 5. Molecular weight distributions of chitosan products with different times of deacetylation. The solvent was 2% acetic acid with 0.1 M sodium acetate. Values were averages of three replications. $\overline{M}_w$ (squares) and peak molecular weight (circles) refer to the left y-axis; $\overline{M}_n$ (triangles) refer to the right y-axis.

TABLE 4

COMPARISON OF COEFFICIENTS OF DETERMINATION (R-SQUARE) FOR TWO METHODS OF SLUDGE COAGULATION EVALUATION -- BUCHNER FUNNEL FILTRATION vs. SPECIFIC RESISTANCE TEST

Sample[1]	Buchner Funnel Filtration[2]		Specific Resistance Test	
	Intercept Eq.	Generalized Eq.	Intercept Eq.	Generalized Eq.
III-A	89.1	52.9	97.3	96.6
III-B	84.1	47.7	94.3	92.8
4-74	83.9	61.0	96.5	94.8

[1]Samples described in Bough et al. (1977a).

[2]Data taken from Bough et al. (1977b).

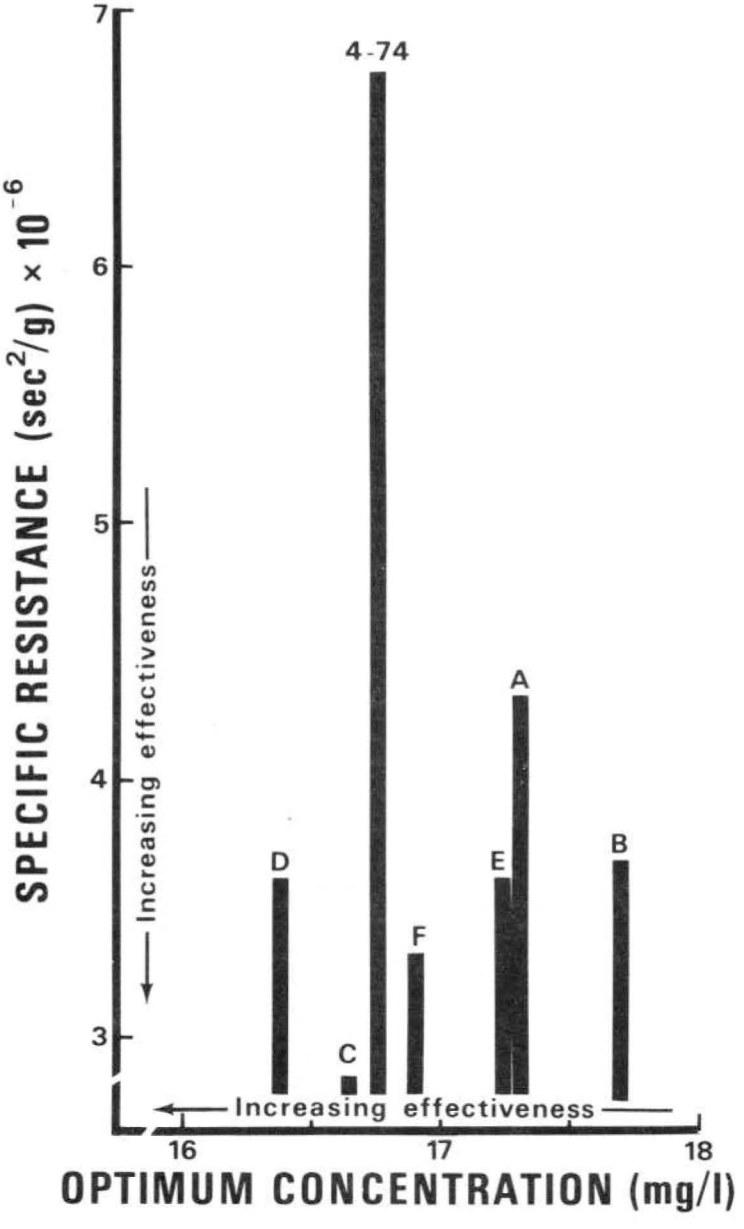

FIGURE 6. Comparison of the optimum dosages and corresponding effectiveness (expressed as specific resistances) of different chitosan samples produced at different times of deacetylation: 30 minutes, 1, 2, 3, 4, and 5 hours (designated by A, B, C, D, E, and F, respectively).

of X and r are also shown in Table 5, which indicate that the correlation coefficient in every case was better than 0.98, that is, these data points were estimated with better than 97% coefficient of determination by the 2nd degree polynomial equations.

These results were further analyzed based on OED values obtained by multiplication of concentration (X) by specific resistance (r) at the optimum dosage for each product. As shown in Figure 7, the OED values decreased to a minimum for the product receiving 2 hours of deacetylation. That is, 2 hours was the optimum time for hydrolysis of acetyl groups from chitin to produce the most effective chitosan product for the coagulation of suspended solids in the activated sludge suspension. The $\overline{M}_w$ of this product was 995,000 and the $\overline{M}_n$ was 186,000. Statistical analysis to compare OED values of different samples by Duncan's Multiple Range test at the 5% level showed (Table 5) that the chitosan sample produced by deacetylation for 2 hours gave treatment significantly better than those samples deacetylated for 30 minutes or 1 hour. Deacetylation for longer than 2 hours did not significantly change the effectiveness of the products. The commercial product was significantly less effective than all the chitosan samples prepared in this study.

As has been observed by other studies on synthetic polyelectrolytes, an optimum MW usually can be found for a polymer for different waste treatment applications (Yorke, 1973). This optimum MW is not necessarily the highest possible value. Such was the case in this study. The $\overline{M}_w$ for the 0.5 hour sample was 1,487,000, compared to 925,000 for the 2 hour sample, and the $\overline{M}_n$ for the 0.5 hour sample was 332,000, compared to 186,000 for the more effective 2 hour sample. We have obtained results where higher MW chitosan products made in our laboratory were more effective for coagulation of activated sludge than a lower MW commercial chitosan sample (Bough et al., 1977b), but observed the lower MW chitosan to be more effective for coagulating suspended solids in cheese whey than most of the higher MW products (Wu et al., 1977). When the changes in molecular weight and percent deacetylation (which is proportionate to the charge density of free amine groups on the polymer chain) were considered to explain the effectiveness of chitosan products, as shown in Figures 4, 5, and 7, the following observations were made. Longer deacetylation times tended to decrease the molecular size and increase the charge density of a product, although the change in charge density of a chitosan sample was within a narrow range (from 67 to 79%). The effectiveness of a chitosan product increased in the early stages of the deacetylation. After reaching a certain stage where the molecular size and charge density reached an optimum state, the effectiveness reached a maximum. Good correlation was obtained for samples deacetylated for 0.5, 1, and 2 hours by multiple linear regression analysis (Hewlett-Packard, 1976) on the relationship among (a) Ln $(\overline{M}_w)$-L_n (% Deacetylation)-(OED), (b) $L_n(\overline{M}_n)$-L_n(% Deacetylation)-(OED), and (c) L_n(Viscosity)-L_n(% Deacetylation)-(OED). Equations (a), (b), and (c) attempt to correlate effectiveness (OED) with $\overline{M}_w$, $\overline{M}_n$ and viscosity, respectively. Results were as follows:

TABLE 5

SUMMARY OF OPTIMUM DOSAGES (X), CORRESPONDING SPECIFIC RESISTANCES (r), AND OPTIMUM EQUIVALENT DOSAGE (OED) OBTAINED FROM THE INFLECTION POINTS OF THE RESPONSE CURVES OF VARIOUS CHITOSAN PRODUCTS TESTED ON ACTIVATED SLUDGE

Sample	Time of Deacetylation, Hour	Optimum Dosage (X), mg/l	Specific Resistance (r) x 10^{-6}	Average Correlation Coefficient	OED x 10^{-6}	Ranks[1] Based on OED
A	0.5	17.30 ± 1.12	4.32 ± 0.74	0.992 ± 0.004	75.3 ± 16.8	--c--
B	1.0	17.69 ± 0.58	3.67 ± 0.70	0.997 ± 0.001	65.1 ± 14.2	-bc--
C	2.0	16.64 ± 0.93	2.84 ± 1.05	0.989 ± 0.006	47.9 ± 19.9	a----
D	3.0	16.37 ± 0.31	3.61 ± 1.77	0.987 ± 0.011	59.3 ± 29.7	abc--
E	4.0	17.25 ± 0.89	3.62 ± 0.99	0.986 ± 0.020	63.0 ± 20.8	abc--
F	5.0	16.90 ± 0.32	3.32 ± 0.42	0.984 ± 0.012	56.2 ± 8.1	ab---
4-74	--	16.74 ± 0.51	6.75 ± 2.67	0.988 ± 0.006	112.9 ± 6.3	----d

[1]Samples having the same letter rank in the Duncan's Multiple Range Test means there were no statistically significant differences at the 5% level of confidence.

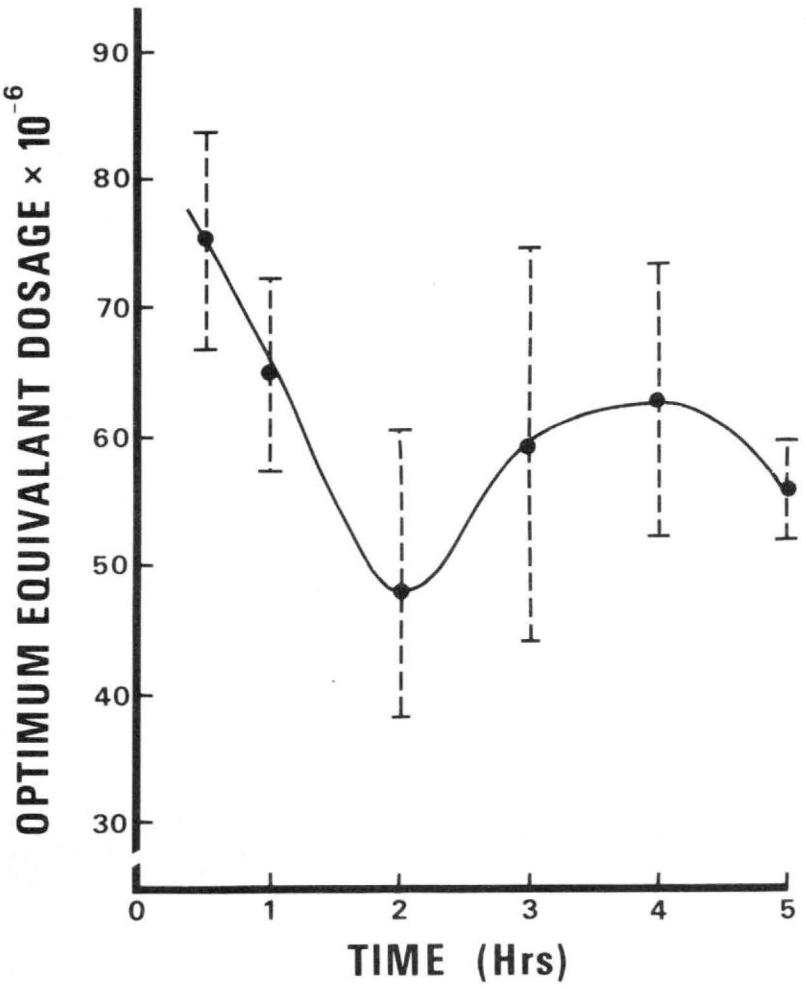

FIGURE 7. Effectiveness of chitosan products produced under different times of deacetylation tested as coagulating agents for the activated sludge treatment. The effectiveness is expressed as the optimum equivalent dosage (OED) based on the specific resistance measurement. Standard deviations of the triplicate runs are also shown.

Model: $(OED) = b_o + b_1 \, LnX + b_2 \, Ln$ % Deacetylation

(a) $(OED) = -2579.1965 + 120.6617 \times Ln \, \overline{M}_w + 223.8484 \times Ln(\%\ Deacetylation)$

$R^2 = 0.7366$, $b_1' = 1.36$, $b_2' = 0.65$

(b) $(OED) = 328.9564 + 53.6497 \times Ln \, \overline{M}_n - 217.7146 \times Ln(\%\ Deacetylation)$

$R^2 = 0.7461$, $b_1' = 0.69$, $b_2' = -0.64$

(c) $(OED) = 51.2725 + 16.2152 \times Ln(Viscosity) + 0.0208 \times Ln(\%\ Deacetylation)$

$R^2 = 0.5327$, $b_1' = 0.74$, $b_2' = 0.03$

where OED was in 10^6X (mg/l)(sec^2/mg) and viscosity was in cps. Based on the R^2 values, molecular weight data ($\overline{M}_w$ or $\overline{M}_n$) correlated well with the effectiveness of the product. This is consistent with the traditional view (Yorke, 1973) that the MW distribution of polymer plays an important role in determining its effectiveness. Based on the beta prime values for correlations (a) and (b), the degree of deacetylation plays an important role in determining the effectiveness of the products. The correlation of OED with $\overline{M}_w$ or $\overline{M}_n$ was better than with viscosity. In previous studies (Bough et al., 1977a; Bough et al., 1977b; Wu et al., 1977), MW determination by HPLC was also shown to be a better method than viscosity measurement to predict the molecular size of the product. Thus, it is suggested that MW determination by HPLC is a better method than viscosity measurement for evaluation of the quality of a chitosan product and predicting effectiveness as a coagulant. From the beta prime values for correlation (c), the % deacetylation data seems not to be an important factor in the correlation between viscosity and effectiveness. It is probably because the viscosity measurement already takes into account the factors that are contributed by the degree of deacetylation, or perhaps the great variation in measurement of viscosity overshadows the influence of percent deacetylation.

In conclusion, the time of deacetylation influenced the size and molecular weight distribution as well as the acetyl content of the chitosan products. For deacetylation times of two hours and longer under the present conditions, the change in acetyl content was slight while the molecular size of the chitosan product kept decreasing. In this study, viscosity measurements correlated well with the weight average MW data. Yet, the MW data correlated better than viscosity with the effectiveness of particular chitosan products measured by the optimal equivalent dosage (OED) for coagulation of an activated sludge suspension.

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