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A Review of the Use of Chitosan for the Removal of Particulate and Dissolved Contaminants

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Abstract: Chitosan has unique properties among biopolymers, especially due to the presence of primary amino groups. Chitosan has been used for the chelation of metal ions in near-neutral solution, the complexation of anions in acidic solution (cationic properties due to amine protonation), the coagulation of negatively charged contaminants under acidic conditions, and for precipitative flocculation at pH above the pKa of chitosan. The coagulation and flocculation properties can be used to treat particulate suspensions (organic or inorganic) and also to treat dissolved organic materials (including dyes and humic acid). This paper will give an overview of the principal results obtained in the treatment of various suspensions and solutions: (a) bentonite suspensions; (b) organic suspensions; (c) anionic dye solutions; and (d) humic acid solutions. Stoichiometry and charge restabilization were determined for the coagulation of humic acid, kaolin, and organic dyes with chitosan, indicating charge neutralization

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as the dominant mechanism for removal. Charge patch destabilization and bridging mechanisms were inferred in other cases, based on the effects of the apparent molecular weight of the chitosan preparations and effectiveness of sub-stoichiometric doses of chitosan. For dye solutions, results showed that color can be removed either by sorption onto solid-state chitosan or by coagulation-flocculation using dissolved-state chitosan; the reactivity of amine groups was significantly increased when dissolved chitosan was used. For humic materials, chitosan can be used as a primary coagulant or as a flocculant after coagulation with alum or other inexpensive coagulants. The influence of the degree of deacetylation and the molecular weight of chitosan on its performance as coagulant/flocculant is illustrated by several examples.

Keywords: Chitosan, coagulation, flocculation, charge neutralization, electrostatic patch, bentonite, dye, humic acid

INTRODUCTION

Chitosan, which is obtained by alkaline deacetylation of chitin, has unique properties among natural polysaccharides. Made up of D-glucosamine and N-acetyl-D-glucosamine units, the protonation of amine groups gives chitosan cationic properties that explain (a) its solubility in the majority of dilute organic and inorganic acids (with the remarkable exception of sulfuric acid); (b) its affinity for anionic compounds (metal anions, organic compounds, dyes) (1). The presence of amine groups leads to the binding of metal cations by complexation or chelation, and after protonation to the binding of metal anions by electrostatic attraction or ion exchange (2). Chitosan has received a great deal of attention in the last decades for the binding of dissolved contaminants by sorption onto chitosan in the solid-state (3), but the literature is much less abundant on the use of chitosan for the removal of particulate and dissolved contaminants using chitosan in the dissolved-state (4–7). Coagulation-flocculation is a key step in the treatment of drinkable or processed water and municipal or industrial wastewaters (8–11). Most coagulation processes use inorganic materials (alum or ferric salts) (12, 13) or cationic synthetic polymers (poly-DADMAC (14), etc.) with the purpose of neutralizing the charge of dissolved or particulate contaminants. Inorganic coagulants can also be used as precipitating agents (sweep floc) (12). The coagulated particles can then be aggregated using flocculants prior to settling (or filtration) (8). Chitosan has characteristics of both coagulants and flocculants: high cationic charge density, long polymer chains, bridging of aggregates, and precipitation (in neutral or alkaline pH conditions). These properties have been exploited for the design of coagulation-flocculation processes applied to the treatment of:

- mineral suspensions: bentonite (15), kaolinite (16);
- organic suspensions: bacteria (17–19), fish processing waste (20), cheese residues (21, 22);
- organic solutions: dyes (23), humic acid (7, 24).

The present work reviews the results we obtained in the treatment of a wide series of simulated effluents containing mineral and organic particulate and/or dissolved contaminants.

The results have been chosen in order to illustrate the different mechanisms involved in the removal of these contaminants:

1. charge neutralization;
2. precipitative coagulation;
3. bridging; and
4. electrostatic patch.

Using chitosan in the dissolved state improves the accessibility and availability of reactive sites compared to the solid state, and comparison of saturation values for sorption and coagulation-flocculation proves, for example in the case of dyes, that the molar ratio between the amine groups (of the biopolymer) and the sulfonic groups (of the dyes) is much greater when using chitosan in the dissolved state (coagulation-flocculation) than in the solid state (sorption) (23, 25–27). The conformation of polymer chains seems to be the key parameter in the comparison of recovery performance.

Other important parameters for the design of coagulation-flocculation procedures using chitosan are related to the physical and chemical characteristics of the biopolymer. Chitosan is a heteropolymer containing both glucosamine and acetylglucosamine units; the deacetylation degree affects the apparent pK_a (and thus solubility and charge) (28). The length of polymer chains (apparent molecular weight) is also an important parameter to be considered for optimizing the use of chitosan in coagulation-flocculation. Several examples are given concerning the impact of physical and chemical characteristics of chitosan.

MATERIALS AND METHODS

Materials

Chitosan samples were donated by Mahtani Chitosan Pvt. (India) and were characterized by their degree of deacetylation (DD) via FTIR with film-state analysis and their apparent molecular weight (MW) via size exclusion chromatography using coupled laser light scattering and refractometry measurements. The weight-average molecular weight and the polydispersity of chitosan samples were measured by SEC-MALLS (size exclusion chromatography coupled with a multi-angle laser light scattering detector). Samples, at a concentration of $1\,\mathrm{mg\ mL^{-1}}$ ($2\,\mathrm{mg\,L^{-1}}$ for low viscosity samples), were dissolved in a 0.2 M acetic acid/ 0.15 *M* ammonium acetate buffer at pH 4.5. The analysis was performed after agitation overnight at room temperature and filtration through Millipore $0.22\,\mu\mathrm{m}$ filter membranes. The samples were injected ($100\,\mu\mathrm{L}$ volume) through a Waters

717+ injector (ERC 3113 degassing unit) using an IsoChrom Liquid Chromatography pump (Spectra Physics) connected to a Protein Pack glass 200SW column and a TSK gel 6000 PWXL column. The flow rate was 0.5 mL min^{-1}. Detection was performed using a MALLS (Wyatt Dawn DSP, equipped with a 5 mW He/Ne laser operating at λ: 632.8 nm) coupled to a differential refractometer (Waters 410). The refractive index increment dn/dc was measured in the buffer with a ScanRef NFT interferometer.

The properties of the samples are summarized in Table 1. The alkaline deacetylation procedure resulted in DD increase but also in a significant change in the viscosity of the resulting solutions (measured with a Brookfield-type viscosimeter) and in the molecular weight of the polymers (MW). Additionally, each sample B1 and C1 was subjected to a supplementary depolymerization procedure to produce samples with lower MW. The MW decreased, going from B1 to B6 and from C1 to C3. The polydispersity (measured by the ratio of MW in weight to MW in number) was variable and high for the C-series, while it remained relatively constant between 2 and 3 for the A and B-series. This means, especially for the C-series, that some polymer chains of lower molecular weight can be present in the solution with possible effects on the coagulation mechanism; the interpretation of experimental data should take into account this parameter.

The polymers were ground and sieved: The smallest size fraction (0–125 μm) of each polymer was collected and used for the preparation of coagulant solutions. The powder was dissolved in an acetic acid solution. To facilitate the preparation of the polymer solutions, chitosan (2 g) was hydrated overnight in demineralized water (98 mL) before 2 g of acetic acid (80%w/w) were added under agitation. The solution was left to stand overnight before being used. The final pH of the solution was close to 4–5.

Organic suspensions were prepared by mixing a given amount of a fine powder of mushroom waste (obtained from an industrial producer of food flavors) with tap water (final mass concentration: 3 g L^{-1}). After fast agitation (i.e. 1000 rpm) for 5 min, the suspension was settled for 20 min in order to collect the supernatant. The suspension was collected under agitation (at 500 rpm) in the upper part of the reactor to obtain homogeneous samples. The electrophoretic mobility of the waste mushroom particles was measured at Penn State University using a Penn Kem Lazer Zee zeta meter (Model 501).

Bentonite was collected from a local site and was ground and sieved. The sieved fraction, particle size below 250 μm, was retained for use in the experiments. Suspensions were prepared to contain 5 g L^{-1} of bentonite. The main characteristics of the tap water used in these experiments are summarized in Table 2. In either case, the initial suspensions were mixed at 1000 rotations min^{-1} for 5 minutes. The suspension was allowed to settle for 20 minutes to remove large non-suspended particles. The supernatant was transferred to a tank that was continuously stirred at 500 rotations min^{-1} for use in the

Table 1. Characteristics of chitosan samples

Sample	Chitosan comm. ref.	Degree of deacetylation (%)	Viscosity (25°C) (cps)	MW_w (g Mol^{-1})	MW_n (g Mol^{-1})	Polydispersity
A	221	78	1500	230700	110000	2.1
B1	222	89.5	740	308300	144800	2.1
B2	232 b2	89.5	262	235200	101300	2.3
B3	232 b3	89.5	186	222000	85400	2.6
B4	242 b4	89.5	72	155000	68700	2.3
B5	242 b5	89.5	28	94300	41300	2.3
B6	242 b6	89.5	18	80100	26100	3.1
C1	233	95	110	182300	84750	2.2
C2	243	95	60	97700	8400	11.6
C3	253	95	12	45100	7300	6.2

Table 2. Main characteristics of tap water

Parameter		Parameter	
Ca^{2+}	$42\,mg\,L^{-1}$ ($1.05\,mmol\,L^{-1}$)	NO_3^-	$3.4\,mg\,L^{-1}$ ($0.06\,mmol\,L^{-1}$)
Mg^{2+}	$20\,mg\,L^{-1}$ ($0.51\,mmol\,L^{-1}$)	HCO_3^-	$140\,mg\,L^{-1}$ ($2.30\,mmol\,L^{-1}$)
K^+	$2\,mg\,L^{-1}$ ($0.82\,mmol\,L^{-1}$)	Cl^-	$9\,mg\,L^{-1}$ ($0.25\,mmol\,L^{-1}$)
Na^+	$24\,mg\,L^{-1}$ ($1.04\,mmol\,L^{-1}$)	SO_4^{2-}	$120\,mg\,L^{-1}$ ($1.25\,mmol\,L^{-1}$)
pH	7.7	Ionic conductivity	$550\,\mu S\,cm^{-1}$

experiments. Vane and Zang (29) reported that bentonite has a negative zeta potential in tap water and under the demineralized conditions that were used in these experiments.

The dye (RB 5) was supplied by Aldrich (U.S.A.). This dye is characterized as a di-azo compound bearing 4 sulfonic groups (Fig. 1). The molecular weight of the dye is 991.82 g mol^{-1}. The commercial salt is supplied as a mixture of the active material and an inert product: the actual dye content is 55%. This must be taken into account for the evaluation of the true molar ratio and sorption capacities (and concentrations).

Humic acid stock solutions were prepared from Aldrich© (AHA) by the following processes: 2 g AHA was placed into 1 L deionized water, the pH was adjusted to pH 10, heated to 115°C for 20 min to hydrate the AHA, cooled to room temperature, filtered using 0.22-μm membranes, and the filtrate was analyzed for dissolved organic carbon (DOC) concentration and absorbance (cm^{-1}) at 254 nm (UV-254). The solution was stored in the dark. AHA prepared in this manner has the characteristics of aquatic fulvic acid.

Kaolin (Georgia© Kaolinite) was used in some experiments. Kaolin was added to deionized water, dispersed using a 20 kHz ultrasound bath, and then other constituents (when used) were added in the following sequence: AHA, $NaHCO_3$, $CaCl_2$ or Na_2SO_4. The pH of the solutions was adjusted with

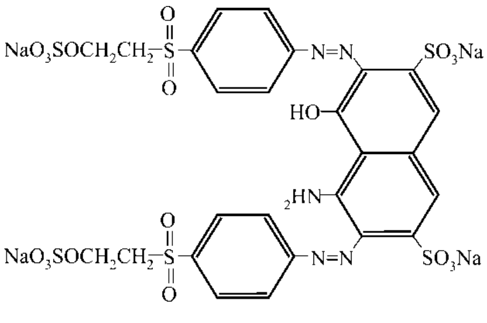


Figure 1. Structure of Reactive Black 5.

0.1 *M* NaOH or HCl. The suspension was mixed for one hour and initial value samples were taken for pH, turbidity, zeta potential (ZP), UV_{254}, or DOC measurements as required. Alum ($Al_2(SO_4)_3 \cdot 14H_2O$) was provided by commercial vendors (2.17 *M* Al) or was prepared from certified reagents (1.00 *M* Al).

Jar-Test Experiments

A jar test apparatus (10409 Flocculator, Fisher Bioblock Scientific) equipped with 75 × 25 mm rectangular mixers was used for studying the coagulation and sedimentation of organic and mineral suspensions and humic acid and dye solutions. The pre-settled suspension was distributed into several beakers each containing 600 mL and a sample was collected for measurement of the initial turbidity. The pH was controlled using 0.01–0.1 *M* HCl or NaOH solutions. Chitosan doses were added to the beakers under agitation (200 rotations min^{-1}). After 3 minutes the velocity was decreased to 40 rotations min^{-1}. The slow agitation was maintained for 20 minutes, then the agitation was stopped and samples were regularly collected in the upper 1 cm of the beaker for turbidity measurements. A blank experiment was performed in the absence of chitosan to evaluate the "natural" decantation of the suspension under each set of experimental conditions. Similar jar-test conditions were used for coagulation studies of kaolin and AHA samples, except that some tests were performed using smaller volumes, more intense rapid-mix conditions, and longer sedimentation times.

Analytical Procedures

Turbidity was measured in triplicate using a HACH DR/2000 turbidimeter, and is expressed in standard formazine turbidity units (FTU). Mean values are reported. Total suspended solids (TSS) were measured in triplicate for some experiments (Standard Methods for the Examination of Water & Wastewater). Correlations between TSS and turbidity were generally greater than 0.99 and therefore FTU was used in this paper as a surrogate parameter for TSS.

The addition of chitosan (dissolved in acetic acid solution) contributes to TOC increase in the solution, which could affect the coagulation mechanism in the case of bentonite suspensions. However, taking into account the low dosage of chitosan in these experiments (systematically less than 1.2 $mg\,L^{-1}$ and in most cases less than 0.5 $mg\,L^{-1}$) the amount of organic carbon introduced remained low enough (less than 0.7 $mg\,L^{-1}$) to make its contribution to coagulation-flocculation performance negligible for bentonite suspensions containing 5 grams of bentonite per liter.

The residual dye concentration was measured by UV-Visible spectrophotometry at a wavelength of 598 nm (UV-VIS spectrophotometer Shimadzu UV-160 A) after pH control. The dye spectrum may be affected by the pH of the solution, so a drop of 3 *M* NaOH solution was systematically added to the samples to maintain a constant pH for the different samples (including calibration samples).

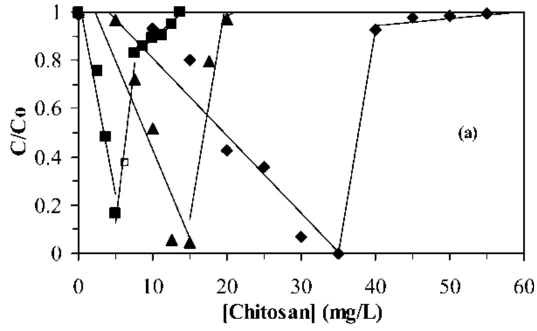
Initial and residual concentrations of AHA were measured using DOC or UV-254 (absorbance at 254 nm). DOC samples were filtered through a 0.22-μm membrane, acidified to pH 2 with 1N HCl, stored at 4°C, purged with N_2 for 10 minutes immediately prior to analysis, and analyzed using a Shimadzu© TOC-5000A.

RESULTS AND DISCUSSION

Mechanisms of Coagulation-Flocculation Involved in the Removal of Dissolved Contaminants

Case of Dye-Charge Neutralization

Reactive Black 5 is an anionic dye bearing sulfonic groups that can interact with protonated amine functional groups in acid solutions. This property has been used for dye sorption on chitosan in sulfuric acid solutions (27). Chitosan flakes dissolved in acetic acid can be used for the coagulation-flocculation of the dye with similar electrostatic interaction mechanisms. The initial pH of the solutions was set at pH 3 and pH 5. The residual concentration of the dye in the upper part of the beakers was analyzed to calculate the efficiency of dye removal (Fig. 1). The pK_a of chitosan depends on the degree of acetylation and the degree of dissociation (28). The intrinsic pK was close to 6.2 at half dissociation for the chitosan samples used in this study. Therefore, when the pH is close to 3, all the amine groups can be considered to be protonated, while at pH close to 5 a small fraction of these functional groups remains unprotonated. For a given dye concentration, increasing the chitosan concentration decreased the residual concentration of the dye up to an upper limit of chitosan concentration. Above this limit concentration (optimum chitosan concentration, OCC) the addition of an excess of chitosan resulted in a re-stabilization of the dye and a decrease in coagulation efficiency. Figure 2 clearly shows that the optimum chitosan dosage (OCC) strongly depended on both the initial pH and the concentration of the dye. This is confirmed in Fig. 3, which plots the OCC as a function of initial dye concentration. For the range of concentrations examined, the OCC increased linearly with the initial dye concentration. It is also important to observe that the OCC provided a precise measurement of the effective chitosan concentration, establishing the stoichiometric ratio between the functional groups of the dye and the biopolymer. Re-stabilization occurred very close to the OCC. Consequently, for practical applications, when the chitosan is added to the coagulation-flocculation reactor it should be continuously delivered in proportion to the instantaneous dye concentration in the



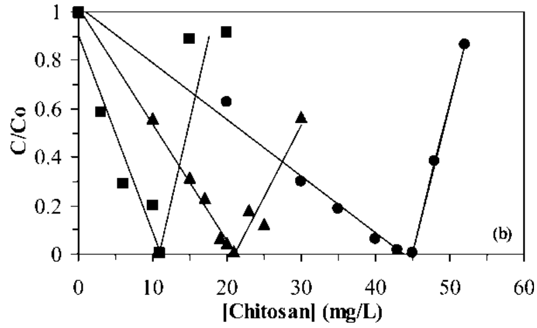


Figure 2. Influence of B1 chitosan concentration on the residual concentration of Reactive Black 5 at pH 3 (a) and pH 5 (b) for different initial dye concentrations (squares: 25 mg L^{-1}; triangles: 50 mg L^{-1}; circles: 100 mg L^{-1}).

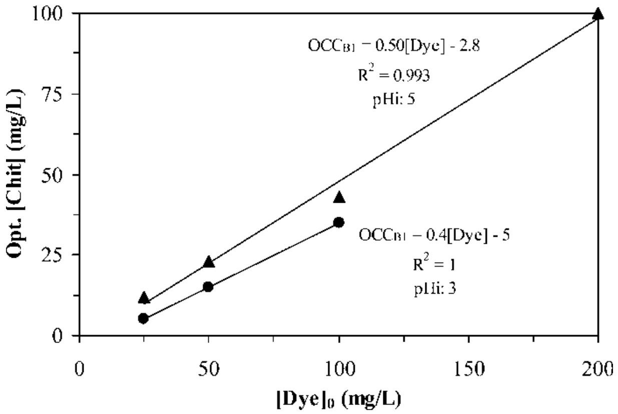


Figure 3. Optimum B1 chitosan concentration (OCC) for Reactive Black 5 removal as a function of dye concentration at pH initial values 3 and 5.

feed (using on-line analysis facilities). The slopes of the re-stabilization sections were systematically steeper than those of the destabilization sections. It should be noted that the optimum chitosan dosages at pH 3 were systematically lower than those required at pH 5. However, the residual dye concentrations were also systematically higher at pH 3 than the equilibrium dye concentrations at pH 5. In solutions prepared at pH 3, there was a relatively high concentration of chloride ions, which may compete with dye anions for binding on protonated amine groups, as well as a higher charge density on the chitosan.

The data shown in Fig. 3 can be used to determine the optimal molar ratio between precipitated dye concentration and chitosan amine groups, [n] (mol dye / mol amine groups). The determination of [n] took into account the water content of solid chitosan, the deacetylation degree, and the true dye concentration (removing the contribution of inert dye adjuvant); and it is considered that all of the chitosan added to the solution can contribute to dye binding. Figure 4 reports [n] versus chitosan concentration for different initial concentrations of dye. This figure contributes to a better understanding of the interaction mechanism between the dye and chitosan, since it provides

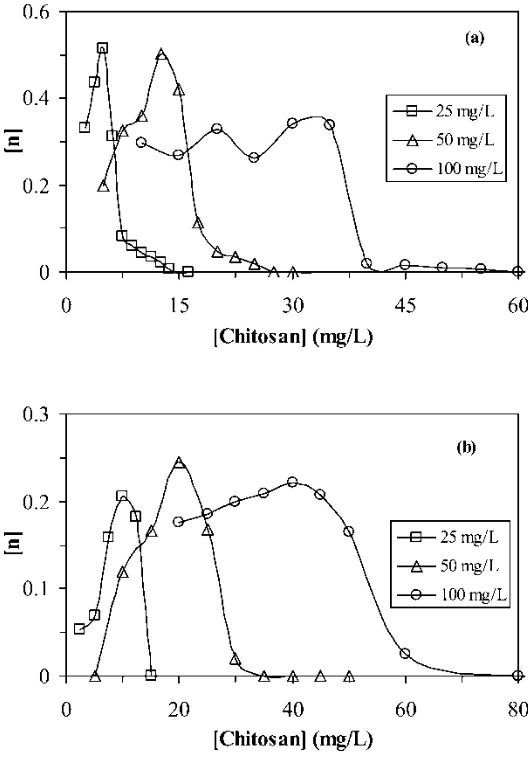


Figure 4. Influence of pH on the maximum [n] ratio (dye/amine molar ratio) for Reactive Black 5 coagulation using chitosan B1 ((a) pH 3; (b) pH 5).

approximate values for the stoichiometric ratio between the dye and the functional groups of the biopolymer. At initial pH 5, the maximum [n] value varied between 0.2 and 0.25 (depending on initial dye concentration). This means that four amine groups were involved in the binding of one dye molecule. Given that the structure of a dye molecule contains four sulfonic groups, a ratio of 0.25 implies that one amine group reacted with one sulfonic group, assuming that all amine groups remained available for interacting with the dye. In this case, the perfect stoichiometry between cationic amine groups and anionic sulfonic groups confirms that charge neutralization is the main mechanism involved in the coagulation-flocculation of Reactive Black 5 using chitosan. At initial pH 3, the maximum molar ratio [n] varied with initial concentration in the range 0.3–0.5: this change is significantly greater than that at pH 5. A lower concentration of amine groups per sulfonic acid group was required at pH 3. The reasons for the decrease in the required amount of chitosan include:

1. a greater charge density of the chitosan at pH 3, and
2. the partial aggregation of dyes (due to the effect of pH), which reduced the number of available sulfonic groups on the dye.

It is important to compare these results for dissolved chitosan with [n] values obtained for the sorption of dyes on solid chitosan (in sulfuric acid media at pH 3). In the latter, the low porosity of chitosan flakes controlled the kinetics of the process and the maximum sorption capacity decreased, resulting in a maximum [n] of 0.12 moles of dye per mole of amine groups. The coefficient [n] in the case of adsorption corresponds to half the value for coagulation using dissolved chitosan. This difference can be explained by:

1. the poor accessibility of amine groups in the sorbent (low porosity), and
2. the limited availability of amine groups because they are engaged in hydrogen bonds within the polymer network with other glucosamine units from the same or vicinal polymer chains.

Case of Humic Acid-Charge Neutralization and Reaction

The pH conditions strongly influenced the removal of humic acid with chitosan (Fig. 5). The lowest chitosan concentration ($2\,\mathrm{mg\,L^{-1}}$) effectively removed AHA until the pH reached 7.5–8. The cationic charge on chitosan is lost for pH values much higher than its pKa, and the efficiency of chitosan to neutralize the anionic charges of humic acid is consequently reduced. When the concentration of chitosan was increased (up to $4\,\mathrm{mg\,L^{-1}}$), the optimal removal efficiency occurred at pH 6–7, consistent with the restabilization of aggregates due to excessive cationic charge at the lowest pH value. For similar reasons, effective coagulation occurred only for higher pH ($\sim$7.5) and a chitosan dose of $8\,\mathrm{mg\,L^{-1}}$, due to an excess of

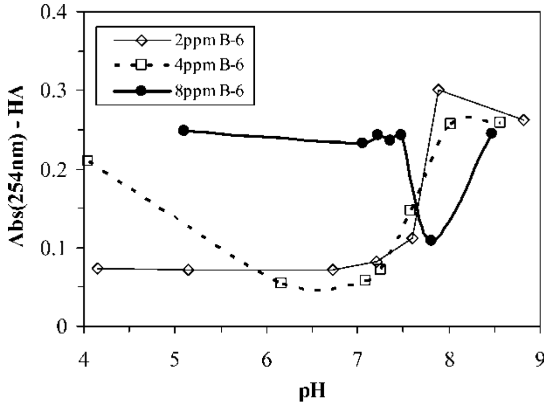


Figure 5. Influence of pH on the coagulation-flocculation of humic acid using chitosan: Effect of chitosan concentration (sample B6) (Aldrich humic acid: $10\,\mathrm{mg\,L^{-1}}$).

cationic charges at lower pH values which re-stabilized the suspension. At pH above 8.5, unprotonated chitosan was not efficient enough to coagulate humic acid, and the precipitation of chitosan was not sufficient to compensate for the loss of the charge neutralization effect. Figure 6 shows the coagulation of AHA (measured at 2545nm) at pH close to 6 for two concentrations of AHA, using two chitosan solutions (B-1 and B-6), and with increasing concentrations of chitosan. The profile clearly shows that the efficiency of the process increased with increasing chitosan concentration up to a concentration limit that depends on HA concentration. Above this concentration limit, the efficiency of the process decreased again. This stoichiometric ratio marks the

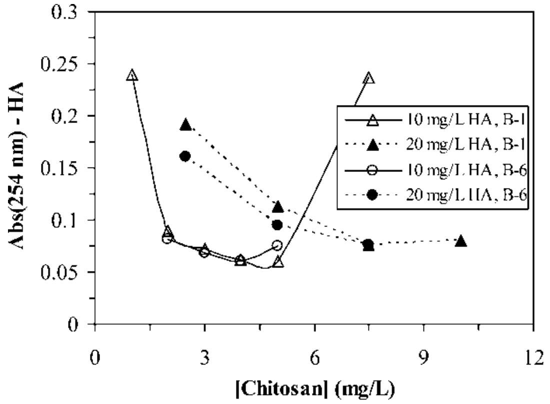


Figure 6. Influence of chitosan concentration (for B1 and B6 samples) at different initial concentrations of Aldrich humic acid (pH: 5.9) on the residual absorbance at 254 nm (17 h settling time).

beginning of the re-stabilization of the suspension, indicating that charge neutralization is a key mechanism in the coagulation-flocculation of humic acid. Figure 6 demonstrates that there was no obvious difference between chitosans B-1 and B-6 for the coagulation of AHA, that the OCC was proportional to the initial AHA concentration, and that re-stabilization occurred. These characteristics are consistent with the charge neutralization mechanism that was also observed for the coagulation of dyes using chitosan.

Figure 7 compares the abatement of the absorbance of the solution at different settling times with changing experimental conditions (i.e. both humic acid concentrations and chitosan concentrations). Again, a minimum concentration of chitosan can be determined, corresponding to charge neutralization, followed by a re-stabilization of the suspension due to excessive chitosan concentration. Increasing the settling time significantly increased the efficiency of the process, and the apparent stoichiometry for initiating the reaction between AHA and chitosan was therefore lower. These results indicate that a relatively slow reaction between humic acid and chitosan also

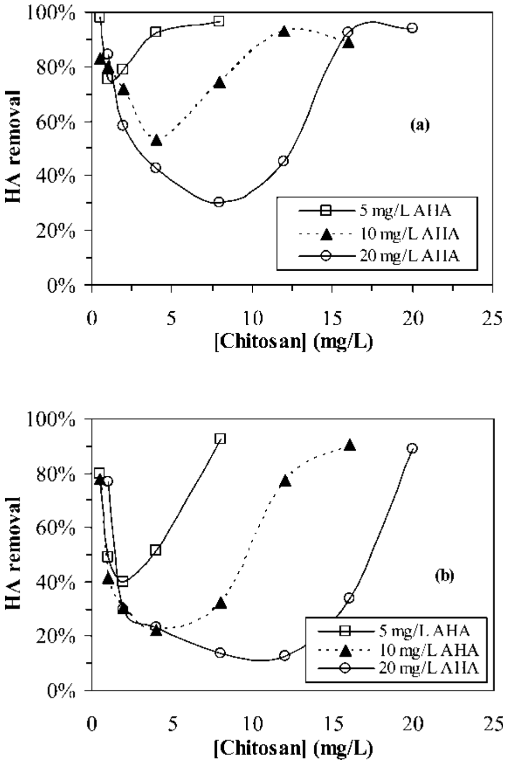


Figure 7. Influence of settling time and B6 chitosan concentration on the removal of Aldrich humic acid (based on absorbance at 254 nm) at pH close to neutral (pH 6–7) and for different initial concentrations of HA: (a) 4 h settling time; (b) 22 h settling time.

contributed to precipitative removal of AHA. Bai et al. (30) used chitosan (in the solid form) for the binding of humic acid by sorption, and then the sorption of lead. Other investigators have reported specific interactions between chitosan and humic materials, and this could explain the decreased sensitivity to stoichiometry with longer reaction time. There was still a linear relation between initial AHA concentration and the chitosan dose required for greatest removal of AHA, as summarized in Table 3. These results indicate that charge neutralization is not the only process involved in the coagulation-flocculation of humic acid solutions; complementary mechanisms that involve specific interactions may be involved such as bridging or charge-patch stabilization mechanisms that have been cited for other systems (31–33).

Mechanisms of Coagulation-Flocculation Involved in the Removal of Particulate Contaminants

Case of Bentonite Suspensions

Very low doses of chitosan were needed for the destabilization and sedimentation of concentrated suspensions of bentonite. Sedimentation was fast, and very low turbidities could be obtained within a few minutes settling time. Figure 8 shows the impact of the composition of the solution (tap or demineralized water) on the coagulation-flocculation efficiency of bentonite, at low chitosan dosage (0.17 mg L^{-1}). In tap water the effect of chitosan was systematically more efficient than in demineralized water. The ionic composition (mainly sulfate ions) could explain some changes in the conformation of the polymer that may contribute to the formation of larger aggregates, especially at pH 7. Effective coagulation was achieved using much lower doses of chitosan than those required for complete charge neutralization of the bentonite. This was most apparent for tap water at pH 7, when the chitosan would not have been completely charged. Simple calculations can demonstrate that complete charge neutralization could not have occurred. Complete ionization of 0.17 mg L^{-1} of B-series chitosan with a degree of deacetylation of 89.5% would produce 10^{-2} meq L^{-1} of cationic charge at pH 5,

Table 3. Coagulation-flocculation of humic acid solutions—chitosan concentrations for greatest removal of AHA (italics) and removal efficiency (in brackets) as a function of settling time and initial AHA concentration

	[AHA] (mg L^{-1})		
Settling time (h)	5	10	20
4	*1* [25]	*4* [47]	*8*
17	*2* [60]	*4* [77]	*8–12* [87]

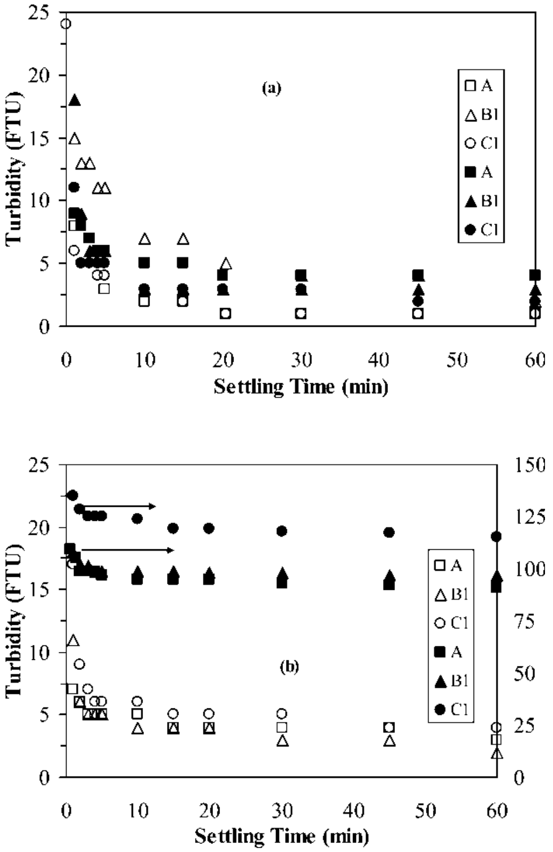


Figure 8. Influence of solution composition on the coagulation-flocculation of bentonite suspensions using three chitosan samples (chitosan dosage: 0.17 mg L^{-1}): Kinetic profiles for tap water (a) and de-mineralized water (b) at pH 5 (open symbols) and pH 7 (closed symbols).

conditions at which it is expected that the chitosan is almost completely ionized (intrinsic pK is about 6.2). The cationic exchange capacity (CEC) was not determined for the investigated mineral sample; however, the literature consulted shows that the CEC, which depends on the mineralogical composition of bentonite, varies between 0.5 and 1 meq g^{-1}. As a consequence, the pre-settled suspension of bentonite ($\sim$2 g L^{-1}) would have possessed about 1–2 meq L^{-1} of negative charge. Thus, none of the experimental conditions were sufficient to achieve complete charge neutralization of the bentonite. This is another argument in favor of the contribution of several mechanisms (e.g. bridging and/or charge patch) to the overall coagulation-flocculation of the mineral suspension. Analogies can be established between the present system and a system described by Ashmore and Hearn

(34, 35) who compared the performance of a series of chitosan samples in the flocculation of model latex particles. At high DD, the optimum chitosan dose for the coagulation of the latex particles was independent of MW. Lower doses of chitosan were required for acidic conditions when compared to slightly alkaline pH conditions, related to the higher charge density on chitosan at lower pHs. For low DD there was improved coagulation with increasing MW. For even lower DD, the charge density of sorbed chitosan decreases and the molecule became stiffer as a consequence of intramolecular hydrogen bonding between $-NHCOCH_3$ and hydroxymethyl moieties of polymer units (from the same or vicinal chains of the polymer). Ashmore and Hearn concluded that coagulation occurred due to charge neutralization and also due to an electrostatic patch mechanism, especially when the positive charges of the chitosan chain were more closely spaced than the center to center distances between the negative charges on the latex surface. Thus, a "mosaic pattern" could be established, with local excess of positive charges in some locations and the original negative charge in other surface locations. Bridging could become more important for lower DD and higher MW chitosan. For these reasons, Ashmore and Hearn recommended the use of high DD under acidic conditions for optimum coagulation efficiencies. They confirmed these results in a more recent paper investigating the same parameters with latex particles of different sizes (35). Increasing the ionic strength of the dispersion medium broadened the flocculation concentration range. Das and Somasundaran (36, 37) also observed a similar synergistic mechanism involving electrostatic patch and bridging for the flocculation of alumina using polyacrylic acid.

A local minimum in residual turbidity was observed for several experimental series during the present work (depending on pH, type of water used for the preparation of the suspensions and type of chitosan). This means that two different mechanisms may be involved in the process, especially when the pH is set at 7. Lower doses of chitosan were required at pH 5 compared to pH 7 (Fig. 9). The effect of pH can be attributed to

1. differences in the protonation of the chitosan amine groups,
2. changes in the conformation of the polymer chain (chain repulsion), and
3. the structure of the clay particles.

Increasing the molecular weight of chitosan, MW, from B6 to B1, improved the efficiency of chitosan at pH 7; at pH 5, the impact of MW was negligible on the residual turbidity of the suspensions.

Case of Kaolin Suspensions

Kaolin suspensions were coagulated using chitosan samples at pH 6.3 (Fig. 10). The efficiency of the process was significantly controlled by the maturation of the suspension: increasing the settling time drastically

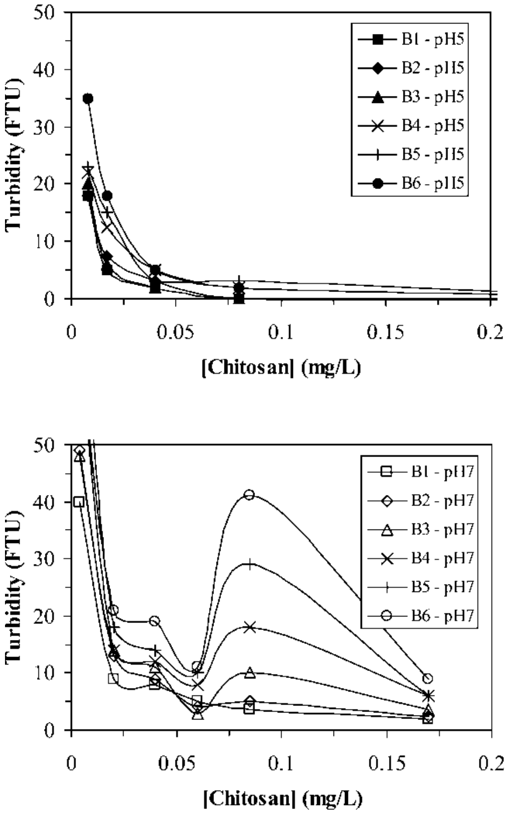


Figure 9. Influence of pH, chitosan concentration and molecular weight on the coagulation-flocculation of bentonite suspension in tap water (Degree of deacetylation of chitosan: 89.5%).

decreased the absorbance of the suspension. It is also interesting to observe that the range of chitosan concentration required for maximum decrease of suspension absorbance was significantly enlarged with increasing settling time. This means that increasing the settling time enabled the system to be managed with greater flexibility as compared, for example, with dye coagulation, where the efficiency of the treatment was very sensitive to an excess of chitosan. This phenomenon of floc maturation is consistent with the results obtained for the coagulation of humic acid (as shown above).

Case of Kaolin-Humic Acid Suspensions

The cases described above considered simple systems (involving a single component in the solution or suspension). It is interesting to study the case of bi-component suspensions, containing both mineral compounds (kaolin,

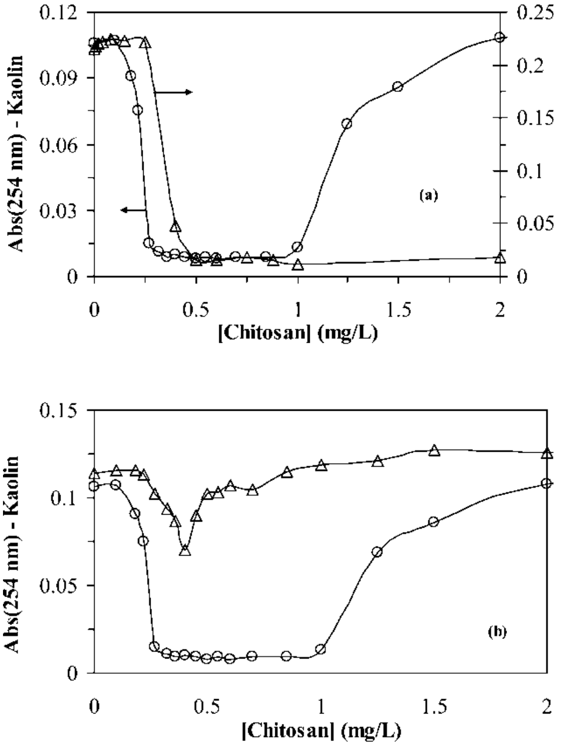


Figure 10. Influence of settling time and chitosan concentration on the coagulation-flocculation of kaolin suspensions (pH: 6.3, 50–100 mg L^{-1} suspensions) using B6 chitosan (absorbance measured at 254 nm) ((a) triangles: 100 ppm Kaolin and 17 h settling time; circles: 50 ppm Kaolin and 22 h settling time–(b) 50 ppm Kaolin, triangles: 4 h settling time; circles: 22 h settling time).

at a concentration of 85 mg L^{-1}) and organic compounds (humic acid, at a concentration of 10 mg L^{-1}): concentrations close to those found in real surface waters. The addition of chitosan as a coagulation-flocculation aid was considered after the addition of alum. The introduction of chitosan significantly decreased the required concentration of alum to achieve a given removal yield (Fig. 11). The profiles were the same for 0.5 and 1 mg L^{-1} chitosan additions: low doses of chitosan were sufficient to improve the efficiency of the alum. Additionally, the molecular weight of chitosan did not change the profile of the curves. This figure also shows that, at an alum concentration of 12 mg L^{-1}, the addition of 0.5–1 mg L^{-1} of chitosan gave a decrease in the absorbance of the suspension comparable to that obtained after filtration of the suspension coagulated with alum alone. The addition of chitosan significantly improved the efficiency of alum for removing contaminants by settling. The chitosan contributes to a better aggregation of

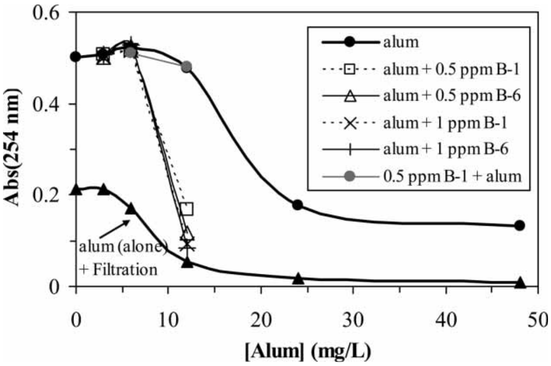


Figure 11. Combined alum/chitosan treatment for the coagulation and flocculation of binary Aldrich humic acid/Kaolin suspensions under selected experimental conditions (pH 6; Kaolin: 85 mg L^{-1}; [HA]: 10 mg L^{-1}; settling time: 3 h).

the flocs, which enhances the efficiency of alum coagulation. In order to optimize the dosage of chitosan, complementary experiments were performed with fixed concentrations of alum and varying chitosan concentrations. Decreasing the concentration of alum required an increased dosage of chitosan to achieve the same results. As the concentration of alum decreased, chitosan had to compensate the deficiency via a dual mechanism (charge neutralization, flocculation aid effect observed in the presence of alum). However, it is noteworthy to observe that the required increase in chitosan dosage is much lower than the decrease in alum concentration. This means that the combined treatment (alum + chitosan) may be very effective for reducing the production of sludge. This is an important criterion for evaluating the process since the cost of chitosan is relatively high compared to conventional reagents; reducing sludge volume may be interesting for adding value to the process. Without alum, the low dose of chitosan was not effective for removing absorbance either by settling or after filtration. The addition of chitosan had almost no effect on removal of the contaminant absorbance after filtration, as shown in the figure below. Overall, the combined use of alum and chitosan with settling was nearly as effective as filtration for removal of the contaminants, given the same coagulant doses. For example, a concentration of alum of 12 mg L^{-1} and a concentration of chitosan of 0.12–0.25 mg L^{-1} followed by settling was quite effective. In the case of suspensions prepared with 85 mg kaolin L^{-1} and 5 mg HA L^{-1} (not shown), similar trends were observed and the required coagulant concentrations were even lower; as an example, alum and chitosan concentrations were reduced to 9 mg L^{-1} and 0.03 mg L^{-1}, respectively. Some experiments were performed in which chitosan was added prior to alum (one example is shown in Fig. 12) and this sequence resulted in decreased removal efficiency after settling.

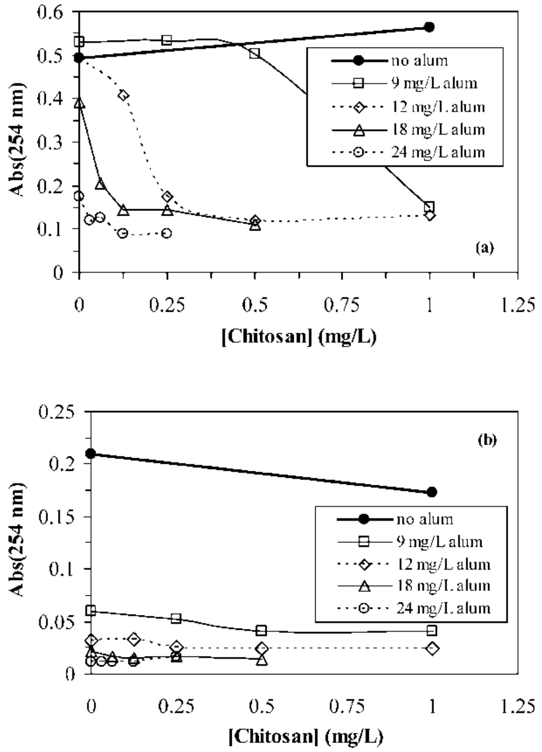


Figure 12. Influence of filtration and B6 chitosan concentration on the absorbance of binary Aldrich humic acid/Kaolin suspension (Aldrich HA [HA]: 10 mg L^{-1}; Kaolin: 85 mg L^{-1}; pH 6; settling time: 3 h) with the addition of different amounts of alum: (a) before filtration; (b) after filtration.

Case of Organic Suspensions

In the case of mushroom suspensions, (used as a surrogate organic particulate contaminant) the pH was a critical parameter, as shown in Fig. 13. Three different pHs were used corresponding to variable degrees of protonation of the polymer: pH 5 for strong protonation, pH 7 for partial protonation, and pH 9 for a virtually un-protonated material. The figure shows that the profiles for turbidity abatement were very close for pH 5 and pH 7, at least in the initial part corresponding to the destabilization of the suspension. In the pH range 3–9, the zeta potential of the suspension was systematically negative with a decreasing trend between pH 2.5 (null value) and pH 3.5 (−10 mV), followed above pH 4 by a slightly decreasing trend (to −20 mV). The addition of protonated amine groups contributed to the neutralization of anionic charges of the organic suspension. The addition of an excess cationic charge led to the re-stabilization of the suspension and a decrease

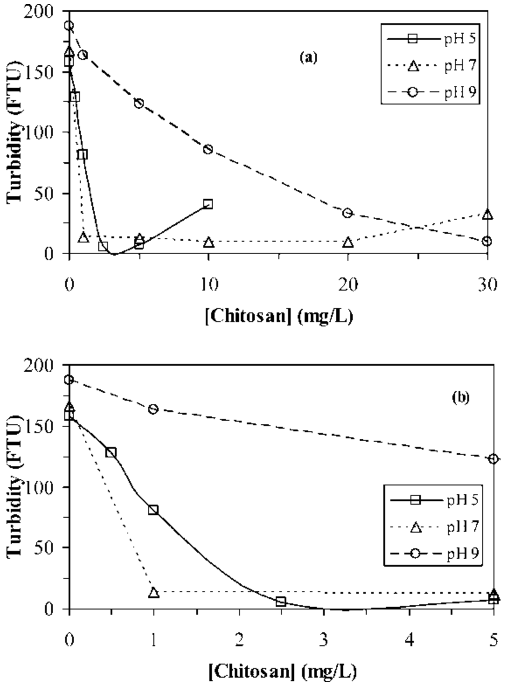


Figure 13. Influence of pH and B1 chitosan concentration on the abatement of turbidity of organic suspensions (mushroom) measured at 30 min-settling time ((a) complete concentration range; (b) low concentration range).

in the efficiency of the process: the optimum concentration range was very narrow at pH 5 (between 2.5 and 5 mg L^{-1}) but the concentration range was wider at pH 7 (between 1 and 20 mg L^{-1}). At pH 5 the optimum concentration was slightly greater than at pH 7 but the coagulation-flocculation was more efficient in acidic solution: the residual turbidity decreased to 5 FTU (compared with 10 FTU at pH 7). The high efficiency of chitosan at pH 7 for turbidity abatement of mushroom suspensions clearly indicates that other mechanisms, complementary to charge neutralization, are involved in the coagulation-flocculation process. At pH 9 (no charge on the polymer) the efficiency of the process increased with increasing concentrations of the biopolymer. A concentration as high as 30 mg L^{-1} was required to reach a residual turbidity below 10 FTU. The interaction of chitosan with organic particulate material may occur due to the entrapment of suspension colloids by the biopolymer during its re-precipitation. We can suspect that, at intermediate pH (i.e. pH 7), both the charge neutralization and precipitative flocculation mechanisms are involved. Actually, the contribution of each individual mechanism depends on the pH of the suspension, which determines the protonation of chitosan and its effect on amine protonation and polymer solubility.

Influence of Physical and Chemical Characteristics of Chitosan

Deacetylation Degree

The deacetylation degree of chitosan is a key parameter determining its electrostatic and rheological properties. The deacetylation degree controls the intrinsic pK of chitosan amine groups, the solubility of the polymer and, together with the molecular weight, may influence the conformation of the polymer (stiffness, charge distribution and charge density). Although the deacetylation degree and molecular weight are characteristics that can be "easily" determined, the identification of the distribution of N-acetyl-D-glucosamine groups requires more complex procedures. The distribution of these acetyl groups can be random- or block- wise. This distribution may affect the interactions between the chains and the degree of their contribution to electrostatic interactions with the colloids via electrostatic patch mechanisms. Figure 14 clearly shows that the deacetylation degree did not significantly change the efficiency of the process in the case of bentonite suspensions when using tap water (whatever the pH, i.e. pH 5 or pH 7), while differences were observed in de-mineralized water at pH 7. The ionic strength decreased the impact of deacetylation degree by a screening effect on charges: the stiffness of the chains is controlled by the electrostatic interactions between the chains, and the ionic strength may reduce the inter chain repulsion. In the case of organic suspensions (Fig. 14), the influence of the degree of acetylation of chitosan was rather limited at pH 5 in comparison to pH 7. At pH 7 the efficiency of the process increased with increasing deacetylation degree (from A to B1). Several experiments performed with variable concentrations of chitosan (in the range 1–5 $\mathrm{mg\,L^{-1}}$) have shown similar trends at pH 5: no

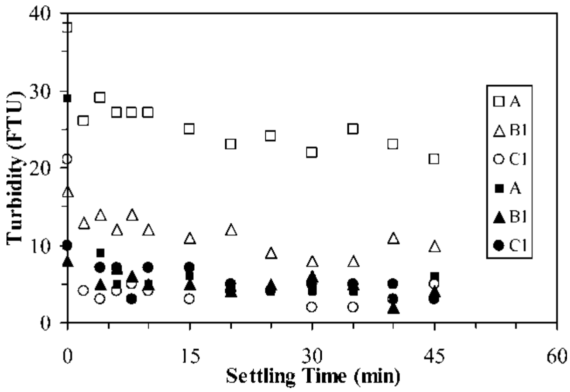


Figure 14. Influence of chitosan characteristics (degree of acetylation) on the coagulation-flocculation of organic suspensions (mushroom wastes) at pH 5 (closed symbols) and pH 7 (open symbols) (chitosan concentration: 5 $\mathrm{mg\,L^{-1}}$).

impact of the deacetylation degree on turbidity abatement (15). At pH 7, a greater amount of chitosan (i.e. 5–10 mg L^{-1}) was necessary to reach the same levels of turbidity abatement as obtained at pH 5, and the efficiency of the process increased with the deacetylation degree, except at low chitosan dosage, where the trend was reversed (in this case the residual turbidity did not conform to required levels, i.e. <10 FTU).

The influence of deacetylation on the samples tested in this study (i.e. 79–85%) was thus negligible, except at pH close to neutral and in suspensions with low ionic strength. The impacts of this parameter on the conformation and the charge of the polymer were easily compensated by the effect of suspension composition. The molecular weight of the coagulant-flocculant would be expected to have a more significant effect.

Molecular Weight

In the case of the coagulation-flocculation of dye (Reactive Black 5), the profiles were very close for samples B1 and B6; however, the chitosan dosage required to reach the lowest dye concentration slightly decreased with decreasing molecular weight (Fig. 15). The figure also shows that the optimum molar ratio, [n] (mol dye/mol amine groups), was systematically higher for sample B6 (low molecular weight, around 0.25 mol/mol) than for sample B1 (around 0.2 mol/mol). This means that, for sample B6, the stoichiometry between sulfonic groups of the dye and amine groups of the biopolymer was respected; while, for the sample B1, an excess of amine groups (about 25%) was necessary compared to the stoichiometry. This can be explained by possible interactions between polymer chains (hydrogen bonds between –OH and amine groups of vicinal units on inter- or intra-chains) which limit the availability of amine groups, and result in greater concentrations of chitosan required for the maximum abatement of Reactive Black 5. On the other hand, the molecular weight of chitosan did not affect the coagulation-flocculation of humic acid, as shown in Fig. 6. The more complex and flexible structure of humic acid (HA) may limit the influence of molecular weight. The contribution of other mechanisms such as bridging or charge-patch stabilization in addition to charge neutralization, which was demonstrated by the fact that the required chitosan/HA ratio decreased with increasing concentration of HA (not shown), may explain why the molecular weight had a lower effect on the coagulation efficiency for HA than for Reactive Black 5.

Figure 9 shows that the effect of molecular weight on the coagulation of bentonite suspensions depends on the pH of the suspension. At pH 5, regardless of the concentration of chitosan, the residual turbidities were comparable for high and low molecular weight samples. In the case of pH 7, the efficiency of chitosan for coagulation and flocculation of bentonite generally decreased with decreasing molecular weight: the differences between the series were amplified at intermediate chitosan concentrations (close to 0.87 mg L^{-1}). At these intermediate chitosan concentrations, the efficiency of the process

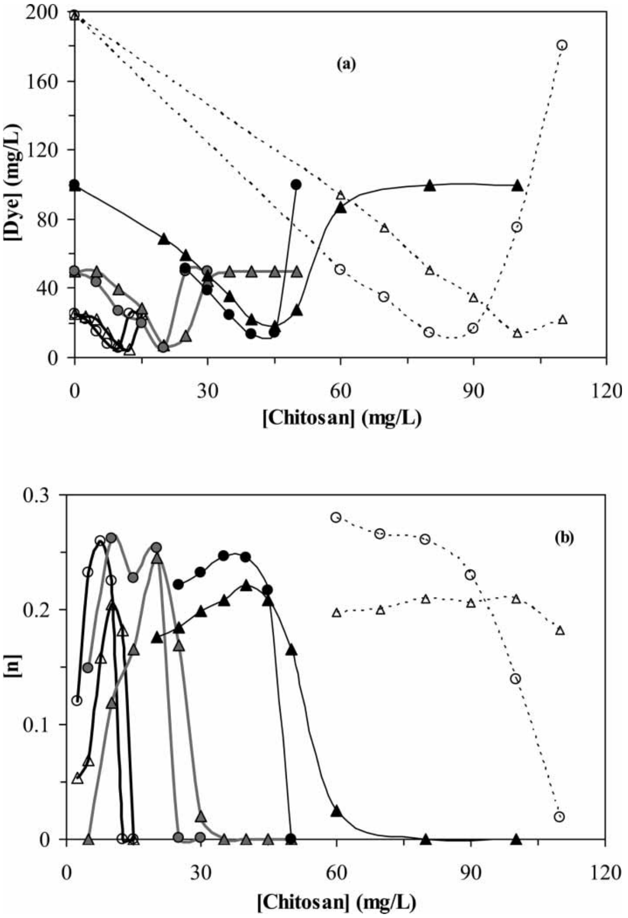


Figure 15. Influence of chitosan molecular weight (samples B1: △, and B6: ○) on the coagulation-flocculation of Reactive Black 5 at pH 5 with different dye concentrations —Effect of polymer concentration on residual dye concentration (a) and [n] molar ratio (b) (Reactive Black 5 concentrations: bold lines: 25 mg L^{-1}; grey lines: 50 mg L^{-1}; thin lines: 100 mg L^{-1}; dashed lines: 200 mg L^{-1}).

reached a local minimum with residual turbidities that increased following the sequence B3 < B4 < B5 < B6 (this effect was not significant for higher molecular weight samples). However, increasing the biopolymer concentration reduced the impact of its molecular weight. The residual turbidity decreased below 10 FTU for all molecular weights in the B-series. In the case of kaolin suspensions, Figure 16 shows that the impact of molecular weight was not very marked. The plots of absorbance of the suspension as a function of the concentration of chitosan were almost superimposed for samples B1 and B6.

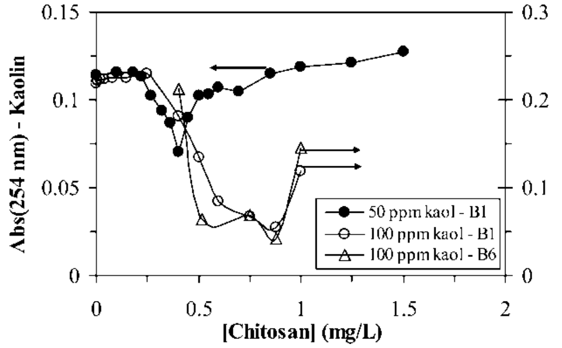


Figure 16. Comparison of B1 and B6 samples for the coagulation-flocculation of Kaolin suspensions in tap water at pH 6.3.

CONCLUSION

Chitosan is very efficient at removing particulate and dissolved contaminants through coagulation-flocculation processes involving several mechanisms such as charge neutralization, precipitative coagulation, bridging and electrostatic patch. Combining these processes (as a function, for example, of pH conditions) enables the design of competitive processes for the treatment of wastewaters or pre-treatment of potable water. This biopolymer offers a promising alternative to the use of mineral reagents (alum salts, ferric salts) or synthetic polymers. The use of a polymer of biological origin, coming from a renewable resource, biodegradable, and thus less aggressive for final discharge in the environment (sludge landfill, dispersion in the aqueous phase of residues) is an important criterion for future developments.

The characteristics of chitosan (degree of acetylation and molecular weight, in the ranges investigated in this study) slightly affected the coagulation-flocculation performance: the molecular weight had more effect on bentonite suspensions and dye solutions than on kaolin and humic acid suspensions. Very low doses of chitosan were required for the treatment of concentrated suspensions of bentonite; these doses were significantly lower when the pH of the suspension was less than the intrinsic pK of chitosan.

In the case of kaolin suspensions, and solutions of dyes and humic acid, the stoichiometry observed between contaminant concentration and required chitosan dose (and the re-stabilization that occurred with excess chitosan) indicated that charge neutralization was preponderant. However, some complementary mechanisms such as bridging or charge-patch stabilization may also contribute.

In the case of binary suspensions, (composed of kaolin and humic acid, 85 mg L^{-1} and 10 mg L^{-1}, respectively) the treatment of suspensions with chitosan was used as a complementary step to pre-treatment with alum. The addition of small amounts of chitosan (for example 0.25 mg L^{-1}) was sufficient to diminish the amount of alum required to half the original value (from 24 to 12 mg L^{-1}).

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