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Effectiveness of chitosan as natural coagulant aid in removal of turbidity and bacteria from turbid waters

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Abstract

There has been considerable interest in the development of natural coagulants such as chitosan. By using natural coagulants, considerable savings in chemicals and sludge handling cost may be achieved. Chitosan, a natural linear biopolyaminosaccharide, is obtained by alkaline deacetylation of chitin. Present study is aimed to examine the effects of aluminium sulfate (alum) as coagulant in conjunction with chitosan as coagulant aid on removal of turbidity and bacteria from turbid waters. These tests were carried out using artificial water and kaoline as model suspensions to represent the wide range of natural turbid waters. A conventional jar test apparatus was employed for the tests. After determining of optimum mixing intensity and duration, alum suspensions were added to the samples and after one minute, the desired doses of natural chitosan were added. In optimum condition, residual Al^{+3} in treated water was less than 0.2 mg/l and meets the EPA guidelines. Turbidity removal efficiency was 74.3- 98.2% by chitosan at a pH 7.0-7.5 for all turbidities. In addition, chitosan significantly reduced the required dosage of primary coagulant 50-87.5%. Bacteria reduction of 2-4 log units (99 - 99.99%) was obtained within the first 1 to 2 h of treatment. Overall results indicate that *E.coli* was removed better than *S. faecalis*. The main effects of coagulation by chitosan on bacteria are enmeshment and stack on the microbial cell surface. We demonstrated that optimal design method is an efficient approach for optimization of coagulation-flocculation process and appropriate for raw water treatment.

Key words: Chitosan, coagulant aid, bacteria removal, water, treatment.

Introduction

Flocculation/coagulation process plays a major role in surface water treatment by reducing turbidity, bacteria, algae, color, organic compounds and clay particles. The processes greatly increase the effectiveness of the latter processes by reducing or eliminating suspended particles that would otherwise clog filters or impair disinfection, thereby dramatically minimizing the risk of waterborne diseases [1, 2].

With aluminium salts, there is always the concern about residuals in the treated water and Alzheimer's disease and, whilst iron salts are a cheaper option, the cost of any imported chemicals can be a serious problem for developing countries. By using natural coagulants, considerable savings in chemicals and sludge handling cost may be achieved [3].

In recent years, chitosan and *Moringa oleifera* coagulants have been applied in water treatment [4]. Chitosan, a natural linear biopolyaminosaccharide, is obtained by alkaline deacetylation of chitin, which is the principal component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters, and cell walls of some fungi such as Aspergillus and Mucor. Chitosan is a weak base and insoluble in water and organic solvents. However, it is soluble in dilute aqueous acidic solutions (pH<6.5), which can convert glucosamine units into soluble form $R\text{-}NH_3^+$. Chitosan is inexpensive, biodegradable and nontoxic for mammals. This makes it suitable for use as a coagulant for a wide variety of suspensions. A chitosan molecule has the ability to interact with bacterial surface, and is absorbed on surface of the cells and stack on the microbial cell surface, forming impervious layer around the cell and blocking the channels [5].

Although, there are many studies on chitosan efficiency as a coagulant in water treatment, but up to the present, special information on the correlation of chitosan and antimicrobial activity is lacking. In this study, the effectiveness of chitosan as a flocculant, when used in conjunction with alum on the removal of turbidity and bacteria was examined at various turbidities.

Materials and Methods

Preparation of artificial water: Synthetic turbid water samples were prepared by adding kaolin into distilled water. Ten grams of kaolin was added to 1 L of distilled water. The suspension was stirred slowly at 20 rpm for 1 h in a jar test apparatus for uniform dispersion of kaolin particles. The suspension was then allowed to stand for 24 h to allow for complete hydration of the kaolin. This kaolin suspension was used as the stock solution for preparation of water samples of varying turbidities for the coagulation tests (Table 1). Three types of turbidities were carried out namely; low turbidity (10-20 NTU), medium turbidity (100-120 NTU) and high turbidity (200-220 NTU) [6, 7].

Table 1. Composition of the artificial water.

Component	Concentration (mg/l)
Alkalinity	100 ± 20
Magnesium hardness	50± 10
Calcium hardness	50 ± 10
pH	7 ±0.5

Preparation of alum solution: Alum solution was prepared by dissolving 10 g alum or aluminium sulfate ($Al_2(SO_4)_3.18H_2O$) in distilled water and the solution was made to 1 L. One ml of this stock solution gives concentration of 10 mg/l when added to 1 L of water.

Preparation of chitosan solution: Chitosan (deacetylated chitin; poly- [1- 4] - β- glucosamine) with minimum of 85% deacetylated prepared from crab shells was obtained from GMA Chemical Company. This was obtained in the form of a pale brown powder soluble in dilute acetic and hydrochloric acids. One hundred milligrams of chitosan powder were weighted into a glass beaker, mixed with 10 ml of 0.1 M HCl solution, and kept aside for about an hour to dissolve. The dissolution process was slow, and some amount of chitosan remained in the form of a thin gel even after this time. It was diluted to 100 ml with distilled water to obtain a solution containing 1.0 mg chitosan per millilitre of solution. The solutions were prepared fresh before each set of experiments [8]. We considered HCl to be a better choice for chitosan preparation from the viewpoints of organic input.

Enumeration of bacteria: *Escherichia coli* (ATCC1339) and *Streptococcus faecalis* (PTCC 1237) were used in all experiments. They were grown using nutrient agar culture in incubator at 37°C for 24 h and kept at 4°C. Confirmation of *E. coli* and *S. faecalis* were carried out by subculturing into Eosin Methylene Blue Agar (EMB agar) and Pfizer Selective Enterococcus Agar (PSE agar) as selective cultures by streak plate method. Enumeration of *E.coli* and *S. faecalis* were carried out with most probable number (MPN index) technique [9].

Experimental procedure: A conventional jar test apparatus, the Phipps & Bird Six-Paddle Stirrer, was employed for the tests, with six 2-L square plexiglas jars, sometimes called Gator Jars [10, 11]. All tests were carried out with 1 L samples in 2-L beakers. Beakers were filled with 1000 ml of the synthetic water, and placed on each slot in a jar tester. Alum was added into each beaker at various doses and agitated at 100 rpm for 1 min. The mixing speed was reduced to 40 rpm for 7.5 min and 20 rpm for 7.5 min. In this stage, the desired doses of chitosan (as coagulant aid) were added. The coagulation pH was kept at 7.0-7.5 by adding 0.1 M H_2SO_4 and 0.1 M NaOH in all coagulation tests. After sedimentation for 20 min, an aliquot of 10 ml was sampled from the mid depth of the beaker and residual turbidity was determined. Turbidity measurements were conducted using turbidimeter (HACH, 2100P). The pH values of samples were measured using pH meter (EUTECH, 1500).

Statistical analysis: The SPSS statistical package (Version 11.5) was used for all statistical analysis. All statistical significance was considered when $P<0.05$. Independent sample test (t-test) was used to confirm the significant differences between the two means. Correlation between two variables was analyzed using bivariate analysis of variance.

Results and Discussion

Determination of optimum pH using alum: Tests were conducted as described at pH values of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0±0.2 using a coagulant concentration of 5 mg/l.The results are presented in Fig.1. The maximum efficiency of turbidity removal is at pH 7.0-7.5 and the residual turbidity drops below 5 NTU. The effectiveness of alum, commonly used as a coagulant, is severely affected by low or high pH. In this pH range, the white flocs obtained were very coarse and settled almost in less than 10 min. The obtained results on optimum pH were in agreement with studies done on alum as coagulant [12]. For clays with a low exchange capacity like kaolinites, the flocculation mechanism by sweeping dominates when the pH is 7.0-8.5 [13].

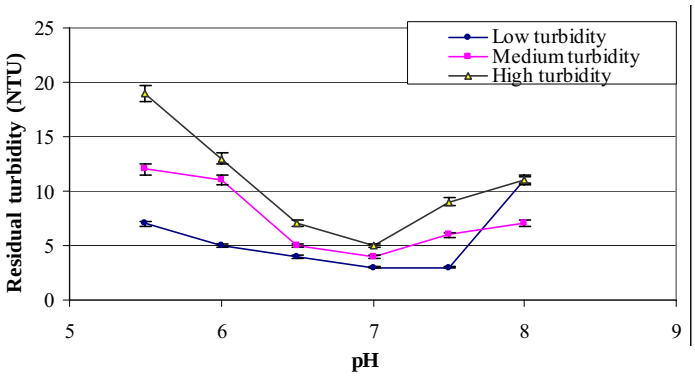

Figure 1. Determination of optimum pH for alum.

Determination of optimum dosage of aluminum sulfate (alum): Results on optimization on alum dosages for low, medium and high turbid water are shown in Fig. 2. Optimum dose of alum for waters with three different initial turbidities is 20, 40 and 20 mg/l, respectively. Above this dosage, the suspensions showed a tendency to restabilise. The lowest dosage with maximal efficiency was found to be 20 mg/l in high turbidity. As initial turbidity of water sample was increased, the required optimum dosage of alum increased. For high turbidity, the optimum dosage of alum decreased. As initial turbidity of water sample was increased, removal efficiency increased. In the present study, it was observed that irrespective of initial turbidity, application of 20-40 mg/l of alum leaves a residual turbidity less than 5 NTU (Fig. 2). WHO recommends that if water is more than 5 NTU, then some treatment to remove turbidity is necessary before the water can be effectively disinfected with chlorine [14]. The turbidity should be measured and if found to be higher than 5, then the next stage is to undertake a simple sedimentation test to establish if and how long it takes for the suspended solids to settle out. This will indicate likely settlement times, which in turn will help with sizing either sedimentation tanks or choosing a coagulation/flocculation based system. At high turbidities, flocs were larger and settling time was lower. The results showed that above optimum dosage, the suspensions showed a tendency to restabilise.

Optimization of chitosan as coagulant aid in conjunction with alum: In order to decreasing residual Al^{+3} concentration in treated water, and possible adverse effects of aluminum in drinking water on human health, chitosan as coagulant aid in conjunction with alum was used. The performances of chitosan in different turbidities are shown in Tables 2 to 4. The optimum dose of alum

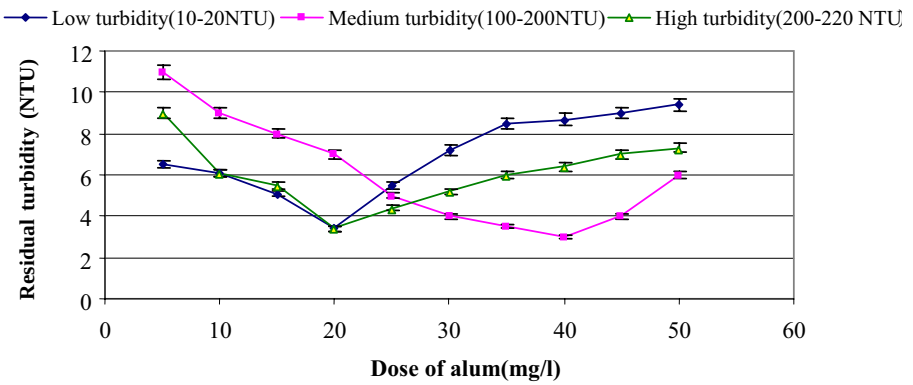

Figure 2. Determination of optimum alum dosage in different turbidities.

Table 2. Determination of optimum dosages of alum combined with chitosan in low turbidity (pH 7.0-7.5).

Dose of alum (mg/l)	Dose of chitosan (mg/l)	Initial turbidity (NTU)*	Final turbidity (NTU)	Removal (%)
0	0.00	17.2	16.2	5.8
10	0.50	15.3	5.6	63.4
10	0.75	14.6	4.8	67.1
10	1.00	15.2	3.9	74.3
10	1.25	15.5	4.4	71.6
10	1.50	15.3	4.7	69.3
10	1.75	14.2	5.0	65.5
10	2.00	15.0	6.5	56.6

*Nephelometric Turbidity Unit.

Table 3. Determination of optimum dosages of alum combined with chitosan in medium turbidity (pH 7.0-7.5).

Dose of alum (mg/l)	Dose of chitosan (mg/l)	Initial turbidity (NTU)	Final turbidity (NTU)	Removal (%)
0	0.00	105	54.0	48.6
5	0.50	101	4.0	96.0
5	0.75	103	6.0	94.2
5	1.00	101	8.0	92.0
5	1.25	108	9.4	91.3
5	1.50	104	5.0	92.0
10	0.50	107	8.3	93.4
10	0.75	103	7.0	93.2
10	1.00	102	8.0	92.2
10	1.25	107	10.0	90.6
10	1.50	102	13.0	87.2

Table 4. Determination of optimum dosages of alum combined with chitosan in high turbidity (pH 7.0-7.5).

Dose of alum (mg/l)	Dose of chitosan (mg/l)	Initial turbidity (NTU)	Final turbidity (NTU)	Removal (%)
0.0	0.0	217	178	18.0
2.5	0.5	209	13	93.7
2.5	1.0	201	9	95.5
2.5	1.5	206	7	95.8
5.0	0.5	214	3.9	98.2
5.0	1.0	215	7	96.7
5.0	1.5	206	10	95.1
5.0	2.0	210	13	93.8

and chitosan when used in conjunction, were 10 and 1 mg/l, 5 and 0.5 mg/l in low and medium turbidities and in high turbidity 5 and 0.5mg/l, respectively. The turbidity removal efficiency in low, medium and high turbidities was about 74.3, 96 and 98.2%, respectively.

It was found that coagulation aid should be added one minute after addition of alum. Poor performance was obtained when coagulant aid and alum were added simultaneously. This was in agreement with studies done on other natural polyelectrolytes as coagulant aids [15]. Results obtained in the laboratory studies showed that chitosan produces appreciable reduction of turbidity between pH 6.5 to 7.5 alone. There was an improvement in the floc size when chitosan was used as a coagulant aid in conjunction with alum. After jar tests, the pH values of treated water was changed about ± 0.1. The use of chitosan as coagulant aid in flocculation process decreased alum dose and the residual turbidity drops to 3.9 NTU. There was an improvement in the floc size when chitosan was used as a coagulant aid in conjunction with alum as compared to either chitosan or alum alone. The pH of solution is a very critical factor in flocculation using chitosan; it is highly effective in a narrow range close to pH 7.The results showed that chitosan did not change pH in water treatment process. These agree well with Divakaran *et al.* [8].

The results showed that the values of the residual Al^{+3} in low, medium, and high turbidities were not more than 0.2 mg/l and meet present standards [16]. In optimum condition (Tables 2 to 4) chitosan reduced the turbidity to below 5NTU without filtration, irrespective of initial turbidity. These agree well with drinking water standards[17]. The total time required for flocculation and settling was less than 40 min. In addition, chitosan significantly reduced the required dosage of alum between 50 to 87.5%, thereby reducing costs of treatment. The performance of chitosan in different turbidities was significantly different in turbidity removal ($p<0.01$). As indicated, the dosage of coagulant and coagulant aid decreased with increasing turbidity. These agree well with earlier studies [18].

It was also found that chitosan did not affect the value of alkalinity. The high content of amine groups in chitosan provides cationic charge at acidic pH and can destabilize colloidal suspension to promote the growth of large, rapid-settling flocs that can then flocculate [19]. Because it is a long-chain polymer with positive charges at natural water pH, it can effectively coagulate natural particulate and colloidal materials, which are negatively charged, through adsorption, charge neutralization, inter-particle bridging as well as hydrophobic flocculation [20].

Effectiveness of chitosan on Escherichia coli and Streptococcus faecalis in turbid waters: Worldwide coliforms have been treated as a reliable microbial tool to determine the microbiological quality of waters and to put in order the water quality guidelines and standards for various modes of water use. *E.coli* is the best coliform indicator of fecal contamination from human and animal wastes. It is more representative of fecal pollution because it is present in high number in fecal material and generally not elsewhere in the environment [21]. Several experiments were carried out to determine the comparative performance of chitosan on *E.coli* and *S. faecalis* in different turbidities. The results are presented in Figs 3 to 8. In this study, water temperature was 20 to 25°C. An inspection of the results indicates that there is a significant difference in removal of *E. coli* and *S. faecalis* at different turbidities. In low turbidity, *E. coli* and *S. faecalis* removal of over 99% was achieved during the first hour of sedimentation in the most experiments. During the first hour of sedimentation, the maximum removal of *E. coli* and *S. faecalis* was 99.9% in medium turbidity and 99.9% in low turbidity, respectively. The artificial water with zero kaoline exhibits almost no bacterial reduction. The rate of orthokinetic flocculation is seen to be first order with respect to the concentration of particles, the velocity gradient and the floc volume fraction.

When the samples were stored during 24 hours; regrowth of *E.coli* was not observed in medium and high turbidity and removal efficiency was achieved to 100%. The number of *E. coli* increased from 2300/100 ml to 43,000/100 ml in low turbidity after 24 hours (Fig. 3). In medium and high turbidities, the number of *E.coli* was

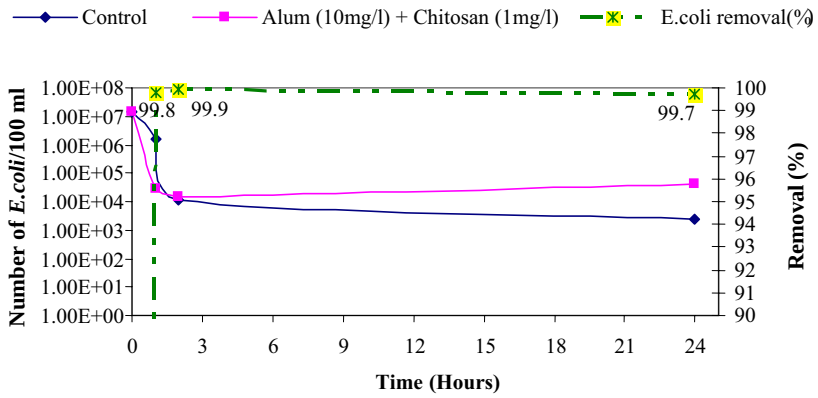

Figure 3. Removal of *E. coli* by alum in conjunction with chitosan in low turbidity.

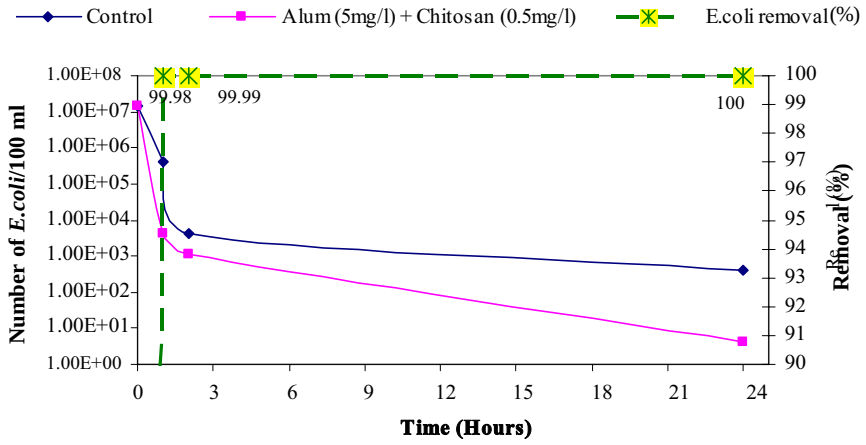

Figure 4. Removal of *E. coli* by alum in conjunction with chitosan in medium turbidity.

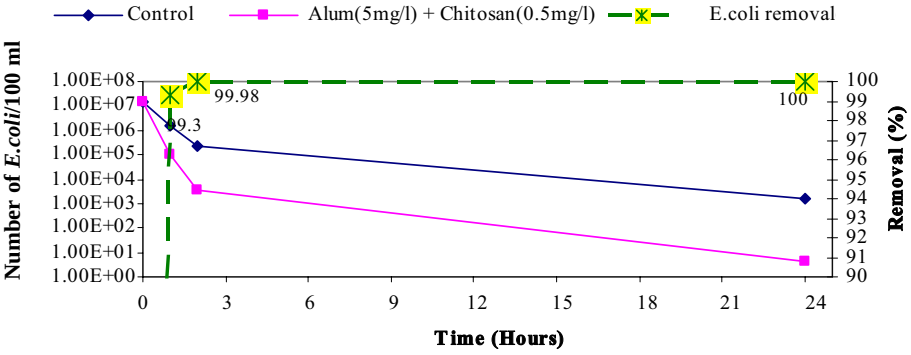

Figure 5. Removal of *E. coli* by alum in conjunction with chitosan in high turbidity.

obtained to below control values and the regrowth was not observed (Figs 4 and 5).

Besides, after 24 hours, removal of *S. faecalis* decreased in medium and high turbidities and achieved to 99% and 99.6%, respectively. The number of *S. faecalis* increased from 4000/100 ml in low turbidity to 460,000/100 ml and 240,000/100 ml in medium and high turbidities after 24 hours (Figs 6-8). In low and high turbidities, the number was below control values and the regrowth was not observed (Figs 7 and 8). It should be noted that the water samples contained no nutrients to support regrowth of *E. coli* and *S. faecalis*. Regrowth of *E. coli* in low turbidity indicates that residual chitosan can be a nutrient source. *S. faecalis* has been retained a longer time in water in comparison with *E. coli*. Therefore, it is suitable bacterial indicator at determining surface water quality and to detection of primitive pollutions. In addition, it can be concluded that the absence of *E. coli* in water treated with chitosan is not necessarily an indication of absence of *S. faecalis*. Despite the difference in size between bacteria and particles, the results of this study showed that removal due to collision was more significant for *E. coli* than for *S. faecalis*. Overall results indicate that *E.coli* was removed better than *S. faecalis*.

It can be concluded that a greater percentage of bacteria was eliminated in higher turbidities. The reduction in the number of bacteria increases with increasing time. The aggregation and, thus, removal of bacteria was directly proportional to the concentration of particles in the suspension. Chitosan as natural coagulant aid showed antibacterial effects of 2 to 4 log reductions. Our findings revealed that water soluble chitosan as a coagulant aid had antimicrobial effects against *E. coli* and *S. faecalis*. Antimicrobial effects of water-insoluble chitosan were attributed to both flocculation and bactericidal activities. A bridging mechanism was reported for bacterial coagulation by chitosan [19].

Chitosan molecules can stack on the microbial cell surface, thereby forming an impervious layer around the cell that blocks

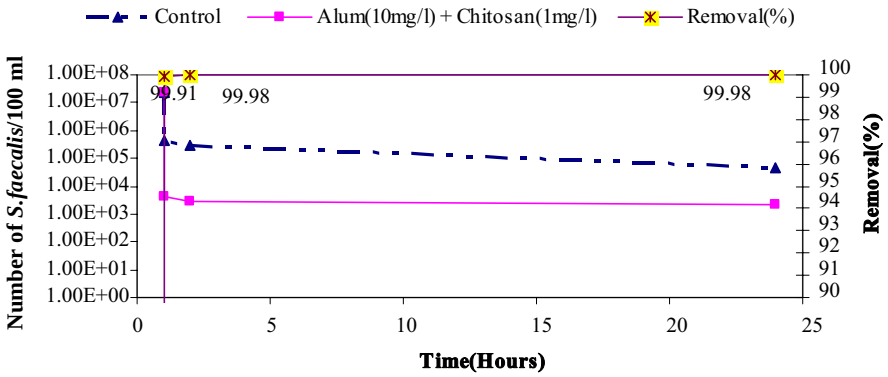

Figure 6. Removal of *S. faecalis* by alum in conjunction with chitosan in low turbidity.

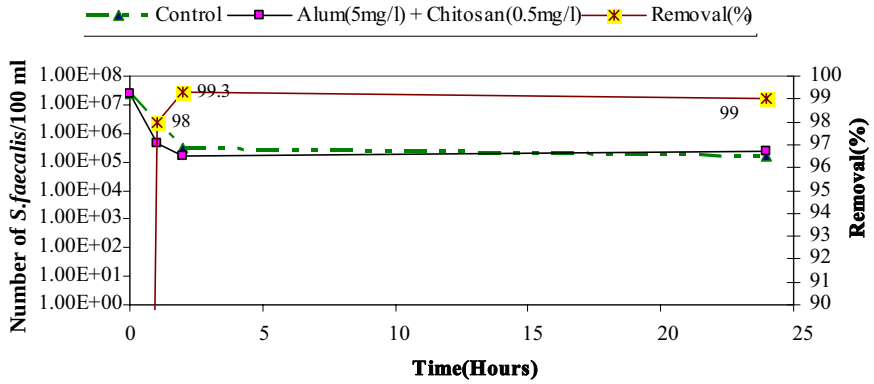

Figure 7. Removal of *S.faecalis* by alum in conjunction with chitosan in medium turbidity.

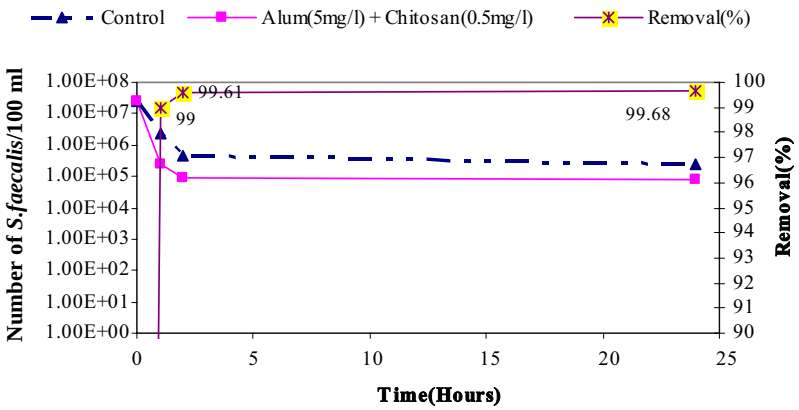

Figure 8. Removal of *S.faecalis* by alum in conjunction with chitosan in high turbidity.

the channels, which are crucial for living cells [5]. On the other hand, cell reduction in microorganisms, such as *E.coli*, occurred without noticeable cell aggregation by chitosan. This indicates that flocculation was not the only mechanism by which microbial reduction occurred. However, further studies are required to establish the true nature of chitosan and the mechanism of antimicrobial action.

Another experiment was designed to check the effect of alum on *E. coli* alone. Regrowth of *E.coli* was not observed for unaided alum after 24 hours. The number of *E. coli* after resuspension of sediment reached to the initial numbers after 24 hours, and showed that the *E. coli* had not inactivated by alum and only concentrated in the sediments.

Conclusions

Residual turbidity was obtained to below standard levels by alum in conjunction with chitosan. Chitosan was not changed pH during water treatment process. It was also found that chitosan did not affect the values of alkalinity. At high initial turbidity coagulation, performance of alum in conjunction with chitosan was much more than at low turbidity. The antibacterial effects were obtained between 2 to 4 log reductions. Flocculation and blocks of the channels were the main mechanisms for antimicrobial effects by chitosan. Regrowth of bacteria was not observed for unaided alum after 24 hours, bacteria were not inactivated by alum, and only concentrated in the sediments. In addition, the absence of *E.coli* in water treated with chitosan is not necessarily an indication of absence of *S. faecalis*. Overall results indicate that *E.coli* was removed better than *S. faecalis*. We demonstrated that optimal design method is an efficient approach for optimization of coagulation-flocculation process and appropriate for raw water treatment.

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