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Straw N-halamines: Evaluation in single and multistage filtration systems

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ABSTRACT

New N-halamines (I-Cl and II-Cl) based on cellulose extracted from rice straw have been evaluated in single and multistage filtration systems against bacteria and viruses. *Escherichia coli* and *Staphylococcus aureus* were used as examples of Gram-negative and Gram-positive bacteria respectively while PRD1 bacteriophage was used as an example for viruses. II-Cl has achieved 9 log reductions in viable counts against *E. coli* in 2 h and *S. aureus* in 1 h while it has achieved 7 log reductions against PRD1 in 5 h. The particle size of prepared materials was modified as well as the flow rate through the filtration systems. The antimicrobial activity of modified cellulose was proved to be comparable to some synthetic biocidal polymers from the same type in similar water treatment systems.

1. Introduction

Straw is used for many applications in rice producing countries; such as producing animal-feed, paper, charcoal, fertilizers and in many other applications (Mansour, Srebric, & Burley, 2007; Suramaythangkoor & Gheewala, 2010; Toor & Beri, 1991). In spite of these enormous amounts of it are subjected to burning-off due to the large remaining stock, which results in risks to health and the environment.

N-halamine polymers have been prepared and evaluated as antimicrobial materials since 1996 by Sun, Chen, and Worley (1996). These materials showed good efficiency as water disinfectants, mainly in water filters (Ahmed, Hay, Bushell, Wardell, & Cavalli, 2008b; Ahmed, Cavalli, Bushell, Wardell, Pedley, et al., 2011). They usually affect microbial cells by halogen exchange through contact, release, and affecting the nature of medium around them (Ahmed, Hay, Bushell, Wardell, & Cavalli, 2009; Ahmed, Wardell, et al., 2011). Converting rice straw into N-halamines may help in two ways: improving air quality in rice-producing countries by reducing the burning process and within the water sector, improving water quality by employment as disinfectants. We have converted cellulosic material extracted form rice straw into N-halamine material (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011). This was achieved by cross-linking cellulose extracted from straw, using amino containing compounds cyclic and acyclic, followed by halogenation (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011). The prepared materials showed good antibacterial effect using agar plates, stirred flask and column methods and could also be recycled several times (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011).

In this work we are extending the study to a full evaluation of the antimicrobial power of such materials in single and multistage filtration systems using different flow rates and different types of microorganisms; bacteria and viruses. The particle size of the prepared materials has also been increased by blending it with sodium alginate and dropping into calcium chloride in order to evaluate its antimicrobial action since large particles will affect the water flow-rate to a lesser extent than closely packed particles (Ahmed, Hay, Bushell, Wardell, & Cavalli, 2010). The multi-stage filtration system includes a sequence of columns containing sand, the non-halogenated form of modified cellulosic material and its halogenated form using, a new approach suggested (Ahmed, Cavalli, Bushell, Wardell, Pedley, et al., 2011). This evaluation will help in forming an opinion over replacing synthetic polymers with this modified cellulosic material for large scale studies. There would be significant reductions in production costs because the starting material is the waste product of rice farming and the added benefit would be to reduce the environmental risk resulting from burning the straw.

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2. Experimental

2.1. Materials

Rice straw was supplied from a farm in Sharkia Governorate, a region in the east Nile delta, Egypt. Sodium hydroxide, calcium chloride, sodium hypochlorite (10% (w/v)) were supplied by Fisher Chemicals, UK. Epichlorohydrin, sodium alginate, and urea were supplied by Sigma–Aldrich Chemicals, UK. Tryptone soya agar, bacteriological agar no. 1, tryptone soya broth, ringer solution (quarter – strength), nutrient Broth and nutrient Agar were supplied by Oxoid Ltd., UK.

2.2. Growth and maintenance of stock cultures

Staphylococcus aureus and *Escherichia coli* K12 were obtained from the University of Surrey culture collection. Primary cultures were maintained on nutrient agar slopes stored at 4 °C. PRD1 (ATCC-BAA-769-B1) and host (*E. coli* ATCC-BAA-769) were sourced from the American Type Culture Collection and were grown/assayed using tryptone soya agar and broth. The titer of the stock culture was 2×10^8 pfu/ml.

2.3. Cellulose extraction (pulping and bleaching)

Rice-straw (10 g, based on dry weight) was ground and refluxed for 1 h in sodium hydroxide solution (17.5%, 100 ml). The resulting material was filtered, washed with distilled water until neutralization and dried at 100 °C for 24 h followed by bleaching using sodium hypochlorite (single stage) (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011; Helmy & Abou-State, 1993).

Raw material showed the following analysis; the ratios of α-cellulose, hemicellulose and lignin were 37.2, 24.7 and 16.2% respectively, while after pulping they were 89.3, 8.6 and 0.8% respectively. After bleaching the ratios of α-cellulose and hemicellulose were 90.3 and 0.6% respectively and degree of polymerization was 762.6 with brightness 87.3% (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011).

2.4. Cross-linking of cellulosic material with urea (I) and cynuric acid (II)

Urea (0.6 g) or cynuric acid (1.9 g) was dissolved in sodium hydroxide solution (100 ml, 0.7% (w/v)). Epichlorohydrin (1.7 g) was added and the reaction heated at 60 °C for 30 min. The cellulosic material (1 g) was added and the reaction continued for an extra 2 h. The resulting material was filtered, washed with distilled water and dried at 95 °C; then cured at 140 °C to increase the cross-linking possibilities within the forming material (I), example in Fig. 1. The prepared material showed the following analysis: (I) = FTIR, υ (cm^{-1}) 1692 (C=O), 3176 (NH), 3430 (OH) and 1080 (C—O), solid state ^{13}C NMR 38, 40–55 (broad band), 65, 77, 85, 90, 108 and 163 ppm, (II) = FTIR, υ (cm^{-1}) 1670 (broad band, C=O), and 3126 (NH), 3423 (OH) and 1010 (C—O), solid state ^{13}C NMR 31, 42, 47, 62, 75, 84, 87, 105, 107, 179 and 182 ppm (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011).

2.5. Chlorination

Modified cellulosic material (I) or (II) was halogenated by soaking 1 g of each in sodium hypochlorite (10 ml, 10% (w/v)) for 1 h at ambient temperature. The resulting halogenated materials (I-Cl or II-Cl) were filtered, washed copiously with distilled water and dried at 40 °C overnight. The halogen content was determined using iodometric titration (Ahmed, Hay, Bushell, Wardell, & Cavalli, 2008a; Chen & Sun, 2006), and the halogenation process was followed by FTIR (Ahmed et al., 2008a; El-Masry, Moustafa, Ahmed, & Shaaban, 2004a, 2004b). (I-Cl) and (II-Cl) showed halogen content of 164 ± 12 and 123 ± 14 ppm respectively while the N–Cl peak on FTIR appears at 775 and 812 cm^{-1} respectively (Ahmed et al., 2008a; El-Masry et al., 2004a, 2004b).

2.6. Alginate bead formation

Sodium alginate was dissolved in water (10 ml) to 3% (w/v) followed by adding (I-Cl or II-Cl, 4% (w/v)), and the mixture was stirred for 30 min. The blend was added drop-wise to a solution of calcium chloride (100 ml, 10% (w/v) $CaCl_2$). The beads (BI-Cl and BII-Cl) were filtered and dried at 40 °C for 24 h. Control beads (BI and BII) were prepared using (I) and (II) (Ahmed et al., 2010).

2.7. The effect of (I-Cl), (II-Cl) and their beads on bacterial and viral viability

A culture of *E. coli* was prepared by inoculating one bacterial colony into 20 ml of nutrient broth in a Universal bottle and incubating for 17 h at 37 °C. From the bacterial suspension, 0.1 ml was transferred to three different Universal bottles containing 10 ml fresh medium. The three bottles were incubated for 17 h at 37 °C, and the number of bacteria determined by viable count. At this time 0.5 g of halogenated material (I-Cl, II-Cl, BI-Cl or BII-Cl) was added to one bottle; 0.5 g of the control material (non-halogenated materials; I, II, BI or BII) was added to the second, and the third was left as a bacterial control. The three bottles were stirred at ambient temperature, and samples from each culture taken for viable count at regular time intervals, Fig. 2a and b. The same procedure was followed to identify the effect of prepared materials on a Gram-positive bacterium (*S. aureus*) (Ahmed et al., 2008b, 2009; Ahmed, Wardell, et al., 2011).

To determine their effect on PRD1 viability, (I-Cl), (II-Cl) or their beads (3 g) was stirred with 30 ml water containing PRD1 (2.1×10^8 pfu/ml). A sample of the suspension (0.1 ml) was taken at time = 0 (immediately after adding the material) and then at timed intervals. Serial 10-fold dilutions of the samples were made in quarter-strength Ringer's solution and the number of viable PRD1 in each dilution was determined using the double-layer agar method (Adams, 1959). Two controls were used; non-halogenated materials and an equal amount of non-treated suspension (viral control), Fig. 2c (Ahmed, Cavalli, Bushell, Wardell, Pedley, et al., 2011).

2.8. Evaluation of (I-Cl), (II-Cl) in water filters on a laboratory scale using different flow rates (single stage evaluation)

(I-Cl), (II-Cl) or their beads (10 g) was packed into a 20 ml glass syringe as a model column. The column was closed, distilled water was added and the column was left overnight to allow the particles to swell. Excess water was removed and the column washed three times with distilled water; 10 ml each time. Microbial suspension (*E. coli*, *S. aureus*) up to 10^5 cfu/ml or PRD1 up to 10^5 pfu/ml) (10 ml) was perfused through the column. The viable counts were followed before and after perfusion. A fresh suspension was perfused again (for 10 cycles for bacteria and 30 cycles for PRD1) through the column and viability was determined after each cycle. A column containing the non-halogenated form of the modified cellulosic materials was used as a control. Bacterial numbers were quantified using the Miles and Misra method (Miles & Misra, 1938) while PRD1 was counted using a double-layer agar method (Adams, 1959). The experiment was performed at 3 different flow rates; 2.5 ml/min, 5 ml/min and 10 ml/min, Figs. 3 and 4.

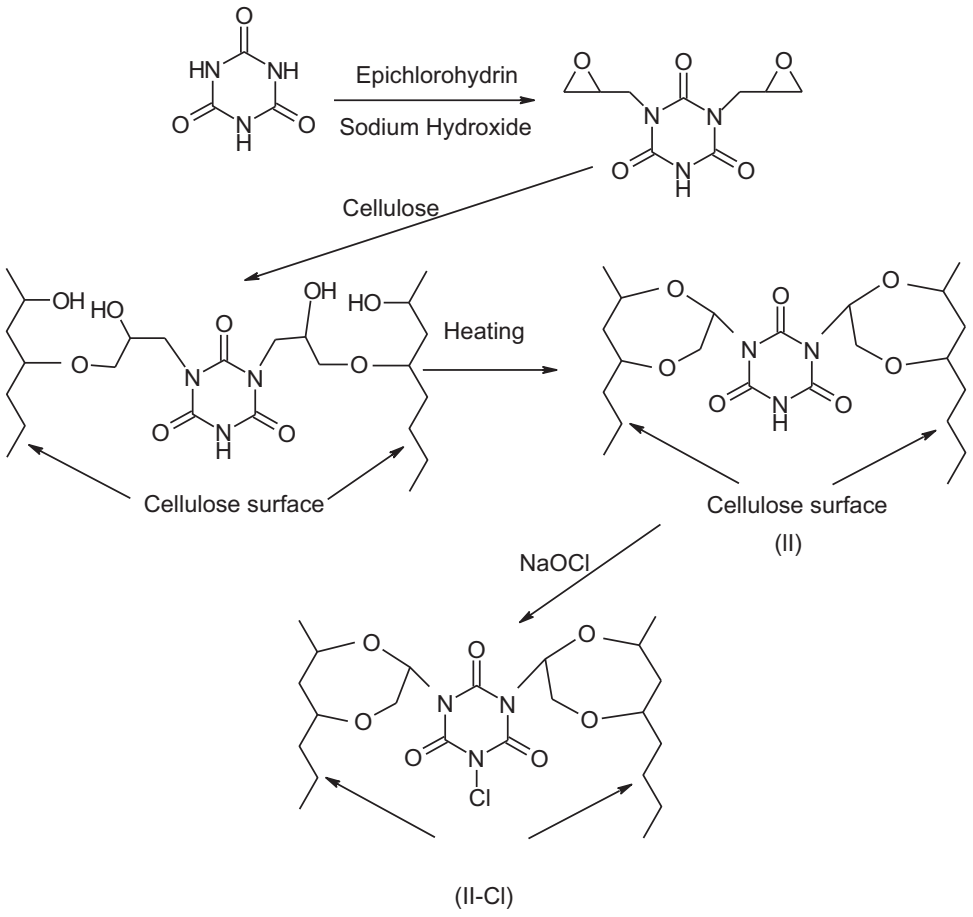

Fig. 1. Cross-linking with cynuric acid.

2.9. Evaluation of (I-Cl) and (II-Cl) in a multistage filtration unit (multistage evaluation)

A unit (on a laboratory scale) formed from three columns; sand (30 g), halogenated materials (I-Cl or II-Cl, 15 g) and non-halogenated material (I or II, 15 g) column was constructed, and challenged with 20 different cycles of microbial suspensions (*E. coli*, *S. aureus* or PRD1). Each cycle was performed using a fresh suspension with bacterial concentrations up to 10^3 cfu/ml or PRD1 concentrations up to 10^4 pfu/ml. The same columns were used for each run to determine the maximum load of the unit. Viable counts were performed before and after perfusing through each column, examples in Figs. 5 and 6. After the third column the level of halogen ions was also measured (Ahmed, Cavalli, Bushell, Wardell, Pedley, et al., 2011).

2.10. Recycling of the multistage system

The previous experiment was repeated 5 times to investigate the recycling possibilities of the multistage system. The unit was washed with 100 ml sodium hypochlorite 5% (w/w) per column to kill any bacteria from the first experiment followed by washing with sterile halogen-free water (100 ml water per column; 10 ml each time). The level of halogen in the washing water at the outlet of each column was measured until no halogen content was recorded. I-Cl or II-Cl column was refreshed by filling it with sodium hypochlorite 10% (w/w) overnight to reload with halogen followed by washing with distilled water. The water was added in portions (10 ml each) and the chlorine content in the outlet was measured using iodometric titration. After cleaning and washing, the second experiment was performed with fresh bacterial or viral suspensions. The same cleaning method was followed in each recycling stage (Ahmed, Cavalli, Bushell, Wardell, Pedley, et al., 2011).

2.11. Re-cycling of prepared materials by full removal of halogen ions

(I-Cl) or (II-Cl) (1 g) was soaked in 20 ml sodium hypochlorite (7.5% (w/w)) overnight. The material was filtered, washed with distilled water (100 ml) and dried at 45 °C for 24 h. The sample was heated in sodium thiosulphate (20 ml, 0.01 M) at 45 °C for 1 h to remove the attached halogen. The material was then filtered, washed with halogen-free water and dried at 45 °C for 24 h. The method was repeated 4 times by charging the material with halogen using sodium hypochlorite then removing it using sodium thiosulphate. After the fourth cycle the biological activity of the I-Cl or II-Cl was studied against bacteria (*E. coli* and *S. aureus*) using a stirred flask method, Fig. 7 (Ahmed, Cavalli, Bushell, Wardell, Pedley, et al., 2011).

3. Results and discussion

Burning rice straw remains a major problem in some rice producing countries (Abou Zeid, El-Fouly, El-Zawahry, El-Mongy, & Abd El-Aziz, 2008; Garas, Allam, & Ragab, 2008). Consuming part of it to produce matrixes for water filters will support two sectors within these countries; environmental protection and water treatment (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011). Toward this we managed to modify cellulosic material extracted from rice straw using cyclic and acyclic amino containing compounds (Ahmed,

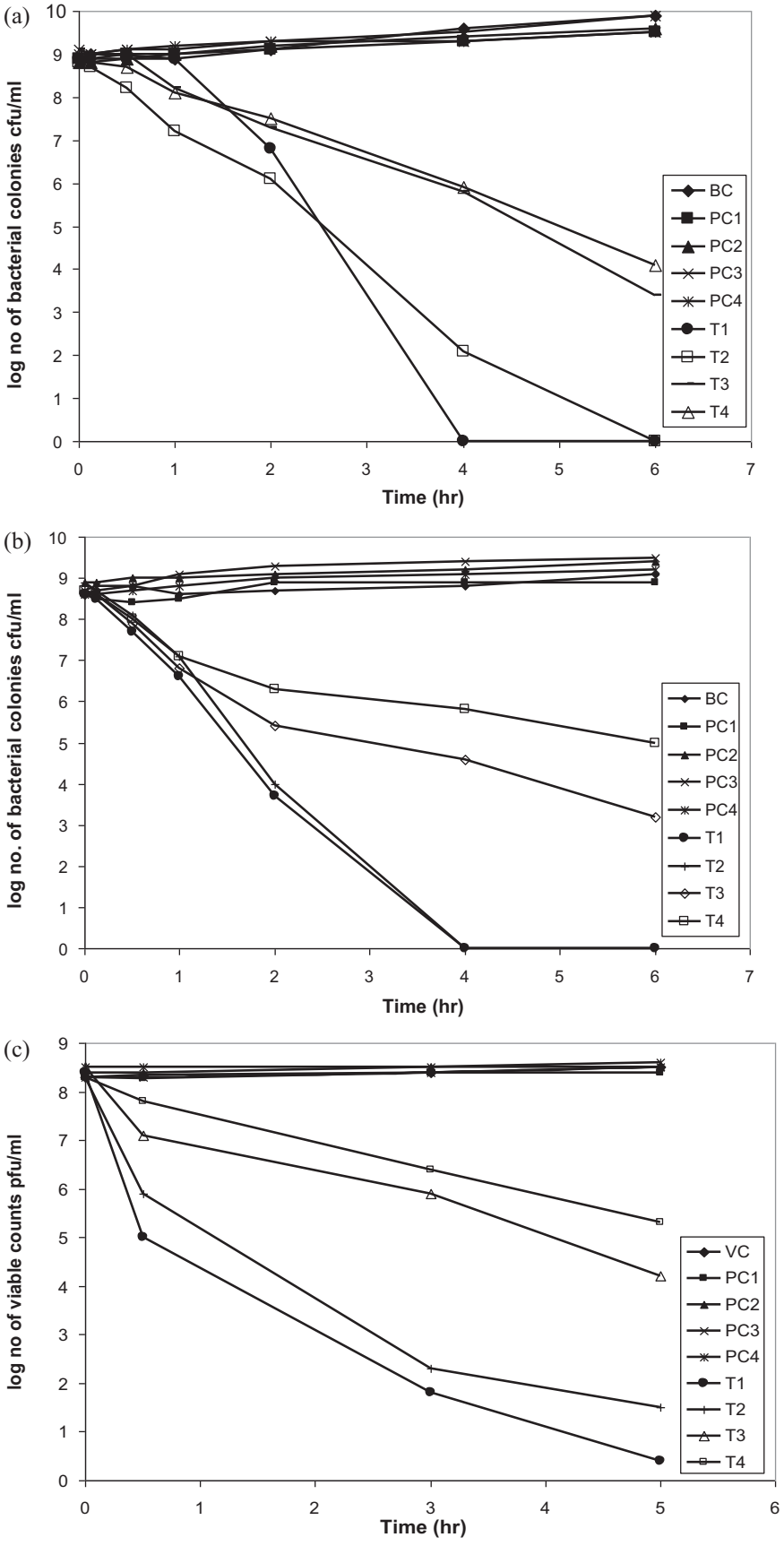

Fig. 2. Effect of (I-Cl), (II-Cl) and their beads on the viability of (a) *E. coli*, (b) *S. aureus* and (c) PRD1. Where BC and VC are the bacterial and viral controls, PC1 and PC2 are I and II, PC3 and PC4 are BI and BII, T1 and T2 are I-Cl and II-Cl and T3 and T4 are BI-Cl and BII-Cl.

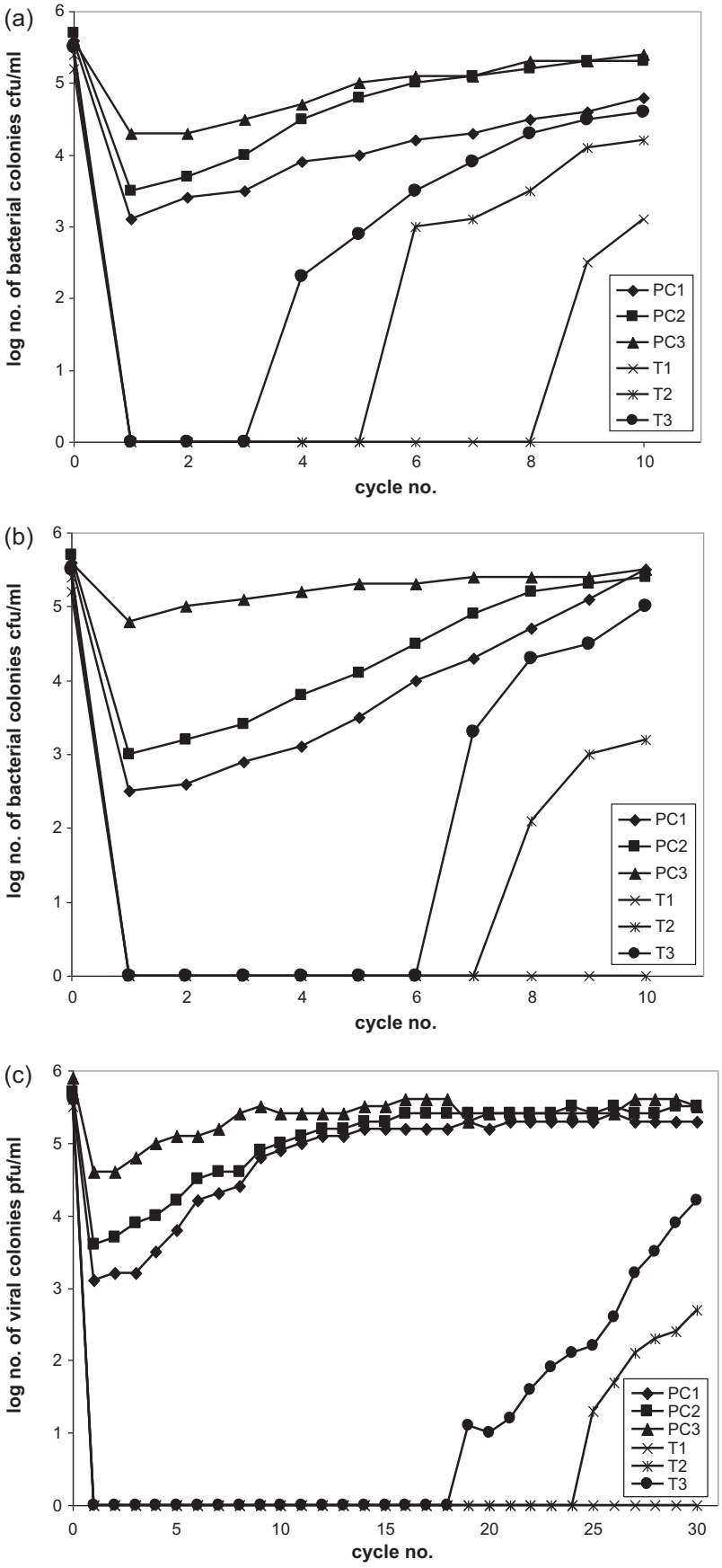

Fig. 3. Evaluation of (I-Cl) in a single stage filtration system at different flow rates against (a) *E. coli*, (b) *S. aureus* and (c) PRD1. Where PC1, PC2 and PC3 are the effect of I at 2.5, 5, and 10 ml/min flow rates respectively and T1, T2 and T3 are the effect of I-Cl at the same rates.

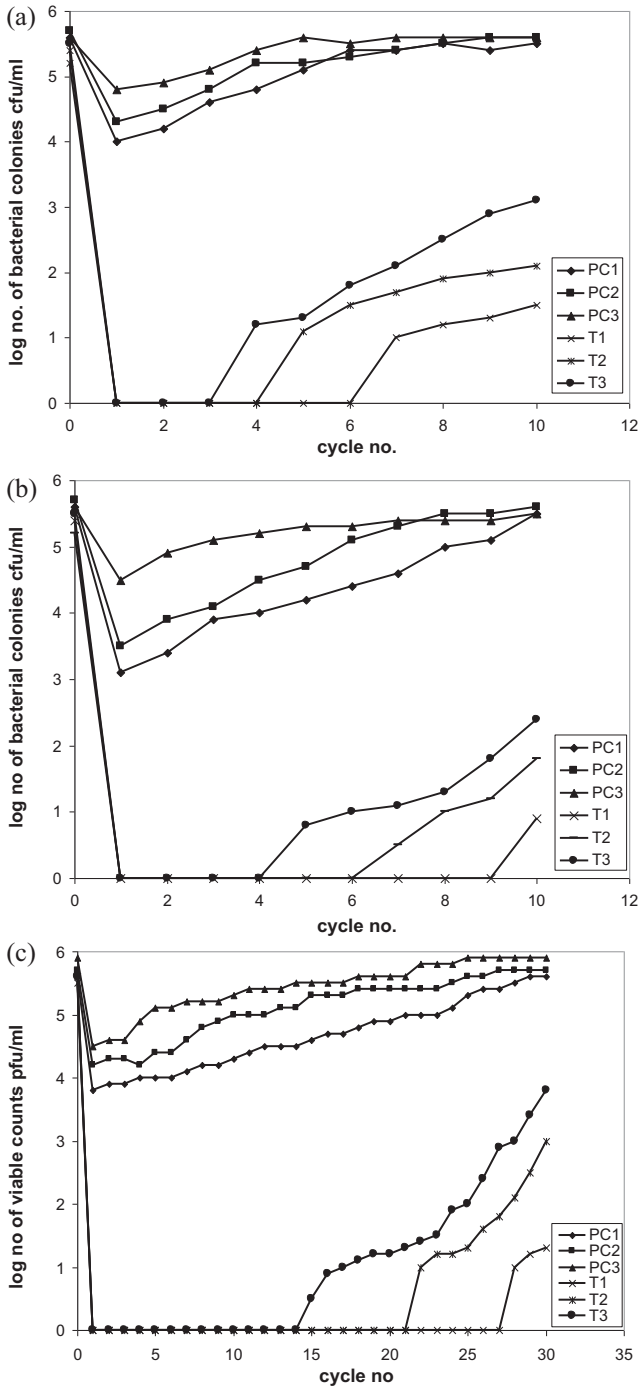

Fig. 4. Evaluation of (II-Cl) in a single stage filtration system at different flow rates against (a) *E. coli*, (b) *S. aureus* and (c) PRD1. Where PC1, PC2 and PC3 are the effect of II at 2.5, 5, and 10 ml/min flow rates respectively and T1, T2 and T3 are the effect of I-Cl at the same rates.

Cavalli, Bushell, Wardell, & Hay, 2011) used as cross-linkers to cellulose in the presence of epichlorohydrin (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011). These amino compounds were selected on the basis that they contain more than two amino/amide/imide function groups; two could work as cross-linkers and the rest could carry halogen (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011). For example: urea, cynuric acid, barbituric acid, semicarbazide and chloroacetamide. The antimicrobial activity of the halogenated form of the modified cellulose materials was studied using agar plate, stirred flask and column method and most of them showed

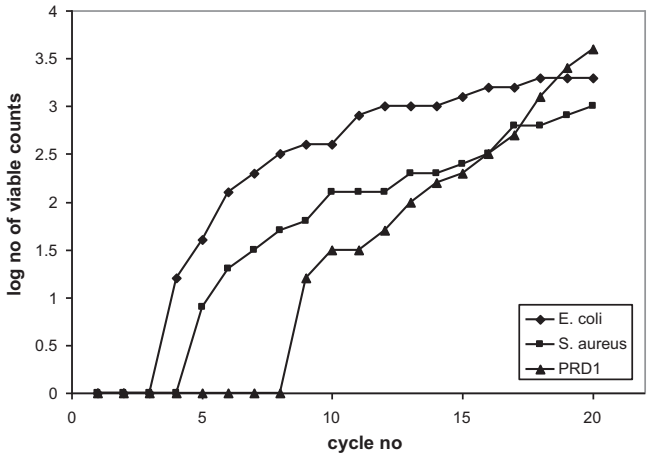

Fig. 5. The viable counts of cells after passing through sand column for 20 cycles.

antimicrobial action actions (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011).

In this work we have selected two examples of these modified materials cross-linked with urea and cynuric acid for further evaluation and show that these materials could be used for filtration purposes and could be transferred to large scale studies in the same way as completely synthetic polymers. Urea was taken as an example for acyclic amino containing compounds while cynuric acid was taken as an example for the cyclic compound. Both of them were reacted first with epichlorohydrin with a ratio 1:2 in basic medium followed by a reaction with cellulose. The materials were then halogenated using sodium hypochlorite to confer some antimicrobial action. At the same time large beads (1–2 mm diameter) were prepared using sodium alginate as a matrix to carry the modified cellulose particles. Enlarging the particles was designed to increase the flow rate through the filtration systems.

Using the stirred flask method it was noticed that I-Cl achieved a 9 log reduction in viable count against both *E. coli* and *S. aureus* in 4 h while it achieved a 7 log reduction against PRD1 in 5 h, Fig. 2. II-Cl performed to similar effect against *E. coli* in 6 h and against *S. aureus* in 4 h while against PRD1 it achieved a 6 log reduction in 5 h. The weakest effect was reported using beads (BI-Cl and BII-Cl), Fig. 2. This could be explained on the basis that using alginate with modified materials reduces the number of the particles (I-Cl or II-Cl) in contact with the microorganisms and at the same time it reduces the amount of released halogen ions to the medium around the cells

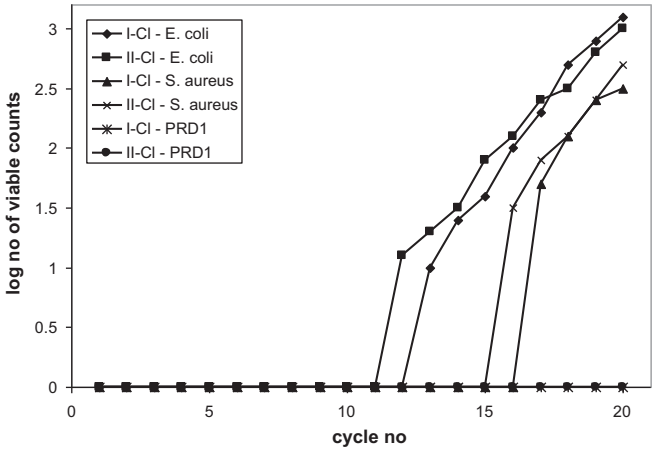

Fig. 6. The viable counts of cells after passing through the second stage (halogenation polymer unit, I-Cl and II-Cl) of the multistage unit.

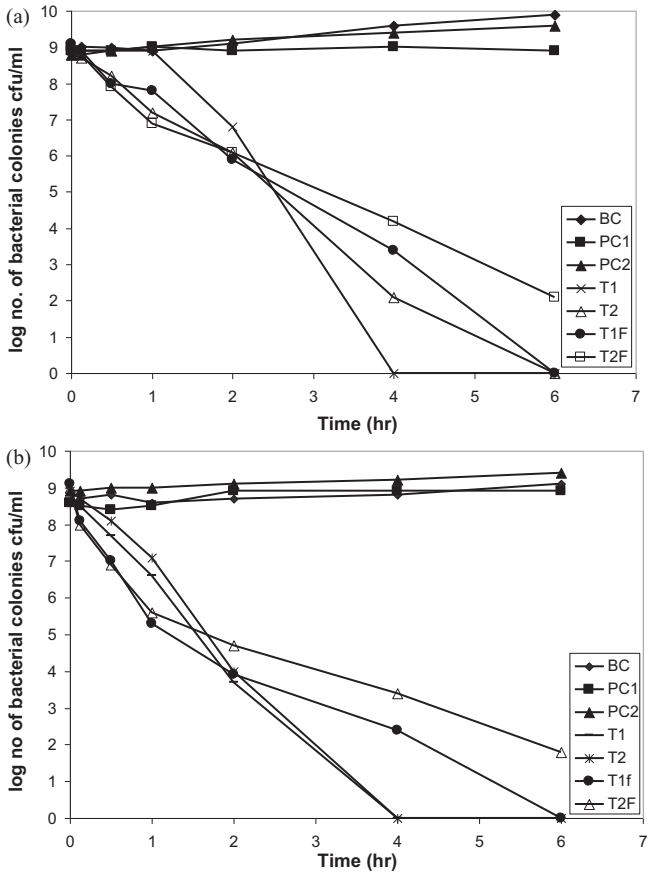

Fig. 7. Investigating the effect of recycled (I-Cl) and (II-Cl) on viability of (a) *E. coli* and (b) *S. aureus*. BC is the bacterial control, PC1 and PC2 are I and II, T1 and T2 are I-Cl and II-Cl and T1F and T2F are recycled I-Cl and II-Cl.

resulting in the reduced effect on viability. Moreover it can be seen that the effect of I-Cl is greater than that of II-Cl as the former has greater halogen content; and also the presence of imide function groups in the cyclic structure confers stability to the halogen ions, compared to the amide function group in an acyclic structure.

I-Cl was previously evaluated for application in water filters against *E. coli* by the action of gravity and its life time and recycling possibility were evaluated (Ahmed, Cavalli, Bushell, Wardell, & Hay, 2011). In this study we have extended the work to cover other bacterial examples such as *S. aureus*, as an example of Gram-positive bacteria, and PRD1 as an example for viruses that have similar characteristics to water-borne viruses. In addition, the effect of flow rate was identified by evaluating the antimicrobial action through perfusing columns at different rates.

It can be seen from Figs. 3 and 4 that increasing the flow rate decreased the antimicrobial action of the filter due to the low contact time. For *E. coli*, reducing the flow rate to 2.5 ml/min increased the ability of the I-Cl column to withstand up to 8 cycles. The same flow-rate upholds the I-Cl column activity against *S. aureus* for 10 cycles and for up to 30 cycles in the case of PRD1. The number of cycles of PRD1 was increased after a significant filtration effect by the materials was noticed. This could be explained on the basis that the hydroxyl and free amide or imide groups in the modified cellulose may form hydrogen bonds with the virus through its protein capsule. This behavior was reported before for *S. aureus* (Ahmed et al., 2008b; Ahmed, Wardell, et al., 2011) and can also be seen from Fig. 3. II-Cl showed similar behavior but its effect was less than that of I-Cl, Fig. 4.

The above results indicated that the prepared materials (I-Cl and II-Cl) have antimicrobial activity and could be used within filtration systems while results from the enlarged beads showed that, although the flow rate was increased, the antimicrobial effect was reduced. The improvement in though-flow may be an advantage in other applications with a lower microbial load such as air filters. The results from I-Cl and II-Cl were sufficiently encouraging to evaluate them in one of our previously suggested systems to produce water free of microbes and halogen ions (multistage filtration system). This will help in forming an opinion over the potential to use this agricultural waste on large scale rather than using other synthetic polymers. The multistage filtration system is formed from 3 columns: sand, halogenated material (I-Cl or II-Cl) and non-halogenated material (I or II). Sand regulates the number of cells going to the main filter (halogenated material filter), Fig. 5. The third column (non-halogenated material column) removes any halogen ions that may contaminate the outlet. The system was charged with bacteria at concentrations up to 10^3 cfu/ml and PRD1 concentrations up to 10^4. Fig. 6 shows that I-Cl column has disinfected the cells received from the sand column for up to 18 cycles in the case of *S. aureus*, 12 cycles for *E. coli* and 20 cycles for PRD1. Similarly, II-Cl achieved 11 cycles for *E. coli*, 15 for *S. aureus* and 20 against PRD1. The results indicated that Both I-Cl and II-Cl achieved excellent filtration and disinfection effects comparable with some of our previously prepared synthetic polymers in similar systems (Ahmed, Cavalli, Bushell, Wardell, Pedley, et al., 2011). This can be explained not only by the degree of halogenation of the material but also on the structure and physical properties. The current material, (I-Cl or II-Cl), contains a large number of hydroxyl groups, beside those already functionalized, s which increases the possibilities of hydrogen bond formation entrapment of the cells.

The system has been recycled several times successfully without losing its antimicrobial action; columns were washed with sodium hypochlorite to kill any remaining viable cells followed by washing with water to remove any remains of adsorbed halogen. The main column (halogenated material column) was recharged with halogen by isolating this column and filling it with hypochlorite. It was recycled up to 5 times without significant loss in its antimicrobial action. But to confirm the recycling possibilities of I-Cl and II-Cl it was important to remove halogen completely from the material before repeating the experiments. These materials (I-Cl or II-Cl) were boiled with sodium thiosulphate to remove all attached halogen ions from the carrying sites followed by recharging again with halogen, using sodium hypochlorite. This process was repeated up to 4 times (charging with halogen followed by removal) and then the effect of these materials on bacterial viability was re-evaluated using a stirred flask method. It can be seen from Fig. 7 that the antimicrobial activity of the prepared materials has been affected by the recycling processes but they still achieve good disinfection activity; for example, the effect of I-Cl on *S. aureus* viability has been changed from a 9 log reduction in 4 h to the same effect but after 6 h.

The above results demonstrate that modified cellulosic material could be used in single and multistage filtration units, providing antimicrobial activity and filtration effects equivalent to or better than some synthetic polymers designed for water treatment. Using such materials in third world countries where rice straw is available as a waste product would be significant in reducing the production costs on commercialization.

4. Conclusion

I-Cl has achieved a 9 log reduction in viable count against both *E. coli* and *S. aureus* in 4 h and a 7 log reduction against PRD1 in 5 h. Similarly, II-Cl performed equal effectively against *E. coli* in 6 h and *S. aureus* in 4 h; while against PRD1 it has achieved a 6 log reduction in 5 h. Both materials have been evaluated in single and multistage

filtration systems. Reducing the flow rate to 2.5 ml/min increased the life-time of both materials. Multistage filtration units based on I-Cl and II-Cl were evaluated and recycled successfully, indicating that the modified agricultural waste could be used instead of synthetic polymers in such applications. Increasing the particles' size using matrixes, such as sodium alginate, reduces the antimicrobial action of the prepared materials. Extra filtration effects were noticed from the modified cellulosic material which was explained by the presence of free hydroxyl groups on cellulose that may result in hydrogen bond formation entrapping microbial cells.

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