Disinfection performance of adsorption using graphite adsorbent coupled with electrochemical regeneration for various microorganisms present in water S.N. Hussaina,c,*, A.P. Trzcinskia, H.M.A. Asghara,c, H. Sattarc, N.W. Brownb, E.P.L. Robertsa,1

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A B S T R A C T

The disinfection performance of the process of adsorption using a graphitic material combined with electrochemical regeneration for a range of microorganisms including bacteria, fungi, yeast and protozoa in a laboratory scale sequential batch reactor is demonstrated. The bacterial species studied were Pseudomonas aeruginosa,Staphylococcus aureus and Legionella pneumophila. A 3.0 log10 reduction in the concentration of P. aeruginosa cells was achieved with the adsorbent that was regenerated at 30 mA cm_2 with 100% regeneration on each adsorption cycle. The process was quite effective in removing S. aureus present in water with a significantly higher reduction in the number of cells (ca. 9-log10 reduction) at relatively low current density (10 mA cm_2). Similarly, L. pneumophila were removed from water with a ca. 7.5-log10 reduction in the number of bacterial cells. The SEM images confirmed the adsorption of L. pneumophila onto the adsorbent and its electrochemical regeneration at 20 mA cm_2 that is considered a refractory pathogen against chlorination. The process was also found to be suitable for disinfecting fungal spores, Aspergillus awamori and yeasts including Saccharomyces cerevisiae and Rhodosporidium turoloides However, the removal of Cryptosporidium parvum from water was not demonstrated successfully. The preliminary results suggest that using a chloride free environment and a relatively high current density could be useful in disinfecting C. parvum.

Introduction The purpose of disinfecting water is the elimination of pathogens that are responsible for waterborne diseases. Chlorination is the most commonly used chemical method of water disinfection which is effective for removing a range of microbial pathogens[1].However, chlorine has been identified as a source of potentially toxic disinfection by-products. It reacts with several organic impurities in water and converts them into trihalomethanes and other halogenated hydrocarbons [2]. Furthermore, significant hazards are associated with the transport and storage of chlorine. In this context, alternative water disinfection technologies have been developed that include chemical and physical processes. Chemical methods employ disinfectants such as ozone [3], chlorine dioxide [4], bromine [5], iodine [6], copper [7] etc. Thermal treatment, ultraviolet irradiation [8], ultrasonication [9], pulsed electric fields irradiation [10] and reverse osmosis [11] are the major physical methods of water disinfection. Amongst physio-chemical systems including photocatalysis using titanium dioxide [12] and photodynamic disinfection [13], electrochemical disinfection of water [14] has emerged as a promising alternative to chlorine providing both primary and secondary disinfection. Electrochemical disinfection has the potential to be developed as a cost effective and environmentally friendly alternative for the disinfection of water and wastewater [15]. During electrochemical disinfection, the water to be treated is passed through an electrolytic cell which is equipped with a set of electrodes.

The effectiveness of the process depends upon cell configuration, electrode material, electrolyte composition, microorganism, water flow rate and current density [14]. One of the main advantages of electrochemical disinfection is the on-site production of disinfectants; thereby the common drawbacks of chlorination including transportation and storage of hazardous chemicals can be avoided [15]. On the other hand, the high cell voltages due to low electrical conductivity of water and the high capital cost are the main bottlenecks for electrochemical disinfection. Electrochemical disinfection of bacteria adsorbed onto the surface of granular activated carbon (GAC) has already been evaluated [16]. The complete sterilization of the adsorbed bacteria could not be possible without having electrical contact for each GAC particle. However, after the adsorption of bacteria onto the surface of GAC, the electrical contact is disrupted by the formation of a bacterial film on the GAC surface. Greater disinfection could be achieved by utilizing more electrically conductive materials. It was, therefore, anticipated that graphite intercalation compound (GIC) would be effective for electrochemical disinfection as the conductivity of the GIC adsorbent bed has been shown to be over 13 times greater compared with powdered activated carbon [17]. Recently, the disinfection of water by a distinctive process of adsorption using graphite intercalation compound adsorbent with electrochemical treatment has been evaluated [18]. Adsorption of Escherichia coli on the GIC adsorbent was followed by electro-chemical treatment under a range of experimental conditions in a sequential batch reactor. The adsorption of E. coli was found to be a fast process and about 8.5-log10 reduction of E. coli concentration was achieved. It was indicated that further work is required to evaluate the treatment of other pathogens including Pseudomonas, Staphylococcus, Legionellla and Cryptosporidium. Therefore, the present study is focused on the removal of a number of microorganisms including bacteria, fungi, algae and protozoa by the process of adsorption with electrochemical treatment using graphite intercalation compound.

Materials and methods

Adsorbent

The adsorbent used was an unexpanded graphite intercalation compound (GIC) in the form of flakes supplied by Arvia Technology Ltd., UK. The material was non-porous as indicated by Mercury porosimetry and therefore did not acquire internal surface area. However, the BET surface area of the particles was found to be around 1.0 m2 g_1 by nitrogen adsorption technique. Laser diffraction (Mastersizer-2000, Malvern Instruments, UK) have indicated that the mean particle diameter of particles was around 480 mm. All the chemicals used in this work were of analytical grades.

Microorganisms

Bacteria

Three bacteria including Pseudomonas aeruginosa, Staphylococcus aureus and Legionella pneumophila were studied in this work. Pseudomonas aeruginosa: Pseudomonas aeruginosa (P. aeruginosa) is a gram-negative rod shaped free living bacterium that is ubiquitous in the environment [19]. It may cause a variety of infections including endocarditis, osteomyelitis, pneumonia, urinary tract and gastrointestinal infections [20]. P. aeroginoas is frequently found in natural waters including lakes and rivers. However, high concentrations of P. aeruginosa can also be found in swimming pools and hot tubs. This is due to the relatively high temperatures and aeration; both of these factors favour the growth P. aeruginosa [20]. Epidemics have also been reported from exposure to P. aeruginosa in swimming pools and water slides [21]. In addition, it has resistance to many antibiotics and disinfectants [22]. P. aeruginosa used in this work was obtained from School of Chemical Engineering and Analytical Sciences, The University of Manchester. Staphylococcus aureus: Staphylococcus aureus (S. aureus) is a gram positive bacterium usually arranged in grape like irregular

clusters. While it occurs widely in the environment, it is found mainly on skin and the mucous membranes of animals. S. aureus can be released into swimming pools, spa pools and other recreational waters by human contact. S. aureus is one of the main causes of pyogenic infections including boils, skin infections, abscesses, osteomyelities, septic arthritis, endocarditis and food poisoning [23]. S. aureus has been found to be more resistant to chlorination than E. coli or P. aeruginosa [23]. A culture of S. aureus (on nutrient agar plate) was obtained from the School of Chemical and Engineering and Analytical Science, University of Manchester, UK.

Legionella pneumophila: Legionella pneumophila (L. pneumophila) is a gram negative rod shaped bacterium. It is one of the water borne pathogens responsible for about 90% of all the cases of legionnaires, a fatal infectious disease [23]. It occurs naturally in rivers and lakes. However, L. pneumoplila also live in cold storage tanks, cooling towers, fire-fighting equipments and spa baths. Stagnant warm water provides an ideal environment for the growth of this bacterium [24]. Inhalation of contaminated aerosols formed by showers, air conditioning systems and cooling towers can spread the disease. Therefore, it is essential to eliminate L. pneumophila from water systems associated with public usage in order to prevent such outbreaks. L. pneumophila strain ATCC 33152 serogroup 1 was obtained from School of Pharmacy and Bimolecular Sciences, University of Brighton, UK.

Fungi

Fungi occupy a wide spectrum of habitats in animal and plant environments, and they are important both as harmful or useful microorganisms. They can contaminate foods and feeds [25]. By contrast, they are also frequently used in the fermentation industry for the production of organic acids, enzymes, vitamins, and antibiotics [26]. Therefore, water is not a primary route for acquiring human fungal infections. However, some fungi including Fusarium can produce toxic substance in water that are associated with a variety of respiratory, neurological and other systemic symptoms [27]. Low concentrations of some of the fungi present in raw water supplies can pass through both sand filtration and disinfection and thus can occur in drinking water leading to potential health problems [28]. In order to investigate whether adsorption using GIC adsorbents with electrochemical regeneration is effective in disinfecting fungal spores in water, Aspergillus awamori (A. awamori) was selected as a model species for water disinfection. A strain of A. awamori (2B. 361 U2/1) classified by the Commonwealth Mycological Institute as in the Aspergillus niger complex, was obtained from the School of Chemical Engineering and Analytical Science, University of Manchester, UK.

Yeast Saccharomyces cerevisiae: In order to investigate the effectiveness of adsorption using GIC adsorbents with electrochemical regeneration to disinfect yeast in water, S. cerevisiae was selected since this species has been intensively studied as a model eukaryotic organism in microbiology. It was obtained from the School of Chemical Engineering and Analytical Science, University of Manchester, UK.

Rhodosporidium turoloides: A culture of R. turoloids Y4 was obtained from the School of Chemical and Engineering and Analytical Science, University of Manchester, UK. Protozoa Free living protozoa are ubiquitous in natural water environments, but also proliferate in water treatment distribution systems [29]. The protozoa; Cryptosporidium parvum, Cyclospora and Giardia lamblia are of great concern because of their adverse impact on human health. These species may cause symptoms including diarrhea, stomach cramps, nausea and vomiting lasting for longer periods [30]. The C. parvum and G. lamblia are the most resistant forms of protozoa and are found in almost all wastewaters [30]. C. parvum was selected as a model species. It was obtained from EasySeedTM in the form of a kit (Z9ES-C100 EasySeed Cryptosporidium 100) which contained 10 _ 10 vials, each holding 10

oocysts (eggs). One advantage of using this kit is that the cells were gamma irradiated. This means that they are already dead and therefore cannot cause disease, but the eggs are intact. Since they are morphologically stable, they will respond in the same way that living eggs would.

Analysis

Bacteria

The plate count method was used to evaluate the number of viable cells [31]. In this context, a number of serial dilutions of bacterial suspension were made in such a way that 100 mL of a given sample was transferred into an eppendorf tube containing 900 mL of normal saline to give 101 dilution. The contents of the tube were thoroughly mixed on a spinmix vortex (Gallenkamp, UK) for a few seconds. From 101 dilution, 100 mL was transferred to another eppendorf tube which also contained 900 mL of normal saline to give 102 dilution. In this way, a number of dilutions up to 109 were made for the concentrated samples. The agar plates were prepared by adding 28 g of nutrient agar to1 L of ultra-pure water. A petri dish was then marked out into four equal quarters for the incubation of various dilutions of a given sample. For one sample, five drops of each dilution with a total volume of 50 mL (each drop is around 10 mL) were gently dropped onto each quarter of the petri dish, with different dilutions in each quarter. The petri dish with the inoculated sample was then placed in an incubator (Gallenkamp, UK) at 37 _C for 24 h for incubation. The colonies that appeared on the petri dish after 24 h were counted to determine the concentration of bacteria in the original sample as colony forming units per mL (CFU mL_1). The agar plates used to inoculate water samples containing L. pneumophila were prepared by dissolving 12.5 g of charcoal yeast in 450 mL of deionised water. Buffered charcoal yeast extract (BCYE) was used as a supplement for the growth of L. pneumophila in charcoal yeast in such a way that one vial of BCYE (Oxoid) was added per 100 mL of the prepared agar. In all other respects, the procedure was as for the viable count described above for P. aeruginosa and S. aureus.

Fungi

The concentration of A. awamori spores was measured using viable count technique as described above. However, the samples of water containing A. awamori were sporulated on a solid medium consisted of 2% (w/v) whole wheat and 2% (w/v) agar. Yeast In case of S. cerevisiae and R. turoloides, the agar media used for viable count was composed of glucose, yeast extract, peptone, malt extract and agar at concentrations of 20, 10, 10, 6 and 20 g L_1. In all other aspects, the procedure was as given described for bacteria above. Protozoa An ordinary microscope (Olympus, BH-2) was used to investigate the samples of water containing C. parvum during adsorption and electrochemical regeneration. Preparation of microorganism suspension in water Bacteria P. aeruginosa & S. aureus: The P. aeruginosa and S. aureus were cultured separately in a sterilized nutrient broth. The resulting suspension was centrifuged and washed before re-suspension in phosphate buffer deionised water to keep the pH neutral throughout the course of experiments. L. pneumophila: The L. pneumophila was cultured in a sterilized yeast extract broth. The resulting suspension was centrifuged and washed before re-suspension in phosphate buffer deionised water.

Fungi

A. Awamori:

A. awamori was initially stored dry in the form of spores in sand at 4 _C. Prior to experimental work, A. awamori spores were purified, sporulated, and stored on slopes at 4 _C. Cultures of A. awamori were sporulated on a solid medium, which consisted of 2% (w/v) whole wheat and 2% (w/v) agar. The spores were suspended in sterilized saline water (0.9%

NaCl), with some drops of Tween 80 (0.01% v/v) (Sigma–Aldrich1). The fungal spores were harvested by the manual shaking of the flasks with sterile glass beads. The spore suspension was transferred into 1 mL vials using a pipette with sterilized tips. A specific concentration of these spores were added to a model synthetic dilute wastewater with a composition given in Table 1 with the aim to give a realistic wastewater in which fungal spores could exist. In addition, sodium bicarbonate used in this composition would not allow a sudden drop in pH which could affect the viability of A. awamori. This low strength wastewater was similar to the one used by Ref. [32]. Yeast S. cerevisiae: The culture was grown in a broth medium composed of glucose, yeast extract, peptone and malt extract at concentrations of 20, 10, 6 and 6 g L_1, respectively. Incubation was carried out at 30 _C for 48 h. Afterwards, a specific volume of this broth was added to the water to be treated in order to study the viability of S. cerevisiae in a general synthetic medium similar to a brewery effluent which contains glucose, yeast extract, peptone and malt extract. R. turoloides: The culture of R. turoloides was also grown in a broth medium composed of glucose, yeast extract, peptone and malt extract. Similarly, incubation was carried out at 30 _C for 48 h. Afterwards, a specific volume of this broth was added to the water to be treated by adsorption and electrochemical regeneration. Protozoa C. parvum: Four vials of C. parvum were added to 500 mL of deionised water. Prior to this, 2 mL of 0.05% Tween (Sigma– Table 1 Model wastewater used in the experiments for A. awamori. Constituent Concentration (mg L_1) Glucose 250 Peptone 200 Urea 10 Meat extract 140 CaCl2_2H2O 4 MgSO4_7H2O 2 K2HPO4 11 NaCl 7 NaHCO3 300 was added into each vial followed by their manual mixing.

Table 1
Model wastewater used in the experiments for *A. awamori*.

Constituent	Concentration ($mg\,L^{-1}$)
Glucose	250
Peptone	200
Urea	10
Meat extract	140
$CaCl_2 \cdot 2H_2O$	4
$MgSO_4 \cdot 7H_2O$	2
K_2HPO_4	11
NaCl	7
$NaHCO_3$	300

Adsorption kinetics

Adsorption kinetic experiments were carried out by mixing a specific cell suspension with a known amount of GIC adsorbent in a volumetric flask. The microorganism suspension and the adsorbent were stirred on a magnetic stirrer. Samples of water were removed at regular intervals of time and analysed for viable cell concentration. Batch adsorption and electrochemical regeneration A specified volume of a known concentration of microorganism suspension was prepared in deionised water (unless otherwise stated) and mixed with a known quantity of GIC adsorbent in the mini-SBR for 30 min using air from a compressor. After the completion of adsorption, the air supply was turned off and the adsorbent particles were allowed to settle for 2 min. A sample of the supernatant above the settled adsorbent was taken from the cell and analysed as described in section Analysis. In the cathode compartment, 0.3% (w/v) NaCl solution acidified with 5 M HCl (to pH 1–2) (unless

otherwise stated) was added as an electrolyte so that the catholyte was at the same level as the bed of settled adsorbent. Electrochemical regeneration of the settled GIC adsorbent was performed by applying a specified DC current for a fixed duration so that the electrochemical regeneration of the adsorbent could take place. After the completion of regeneration, the current was turned off and a sample of water was again taken from the supernatant liquid for analysis (using the method described in section Analysis). Subsequently, the water present above the regenerated bed and the catholyte were siphoned off separately. The water siphoned from the anode compartment was collected and sterilized before disposal. A measured volume of microorganism suspension of known concentration was added to the mini-SBR and re-adsorption was carried out under identical conditions to the initial adsorption stage. For adsorption and electrochemical regeneration over a number of cycles, the adsorption and regeneration procedure was repeated several times. Samples of GIC adsorbent were collected during adsorption and electrochemical regeneration for SEM analysis. A number of adsorption and electrochemical regeneration experiments were performed in the SBR as shown in Fig. 1. However, so as to circumvent the treatment of large volumes of water contaminated with a microorganism, another SBR was constructed with the same geometry as that shown in Fig.1, however with a reduced electrode area of 20 cm2.

Results and discussion

Bacteria

Pseudomonas aeruginosa

The suitability of the process was tested by performing a series of adsorption and electrochemical regenerations in the mini-SBR following the procedure described in section Batch adsorption and electrochemical regeneration. The water contaminated with P. aeruginosa was prepared in phosphate buffer deionised water according to the procedure described in section Preparation of microorganism suspension in water.

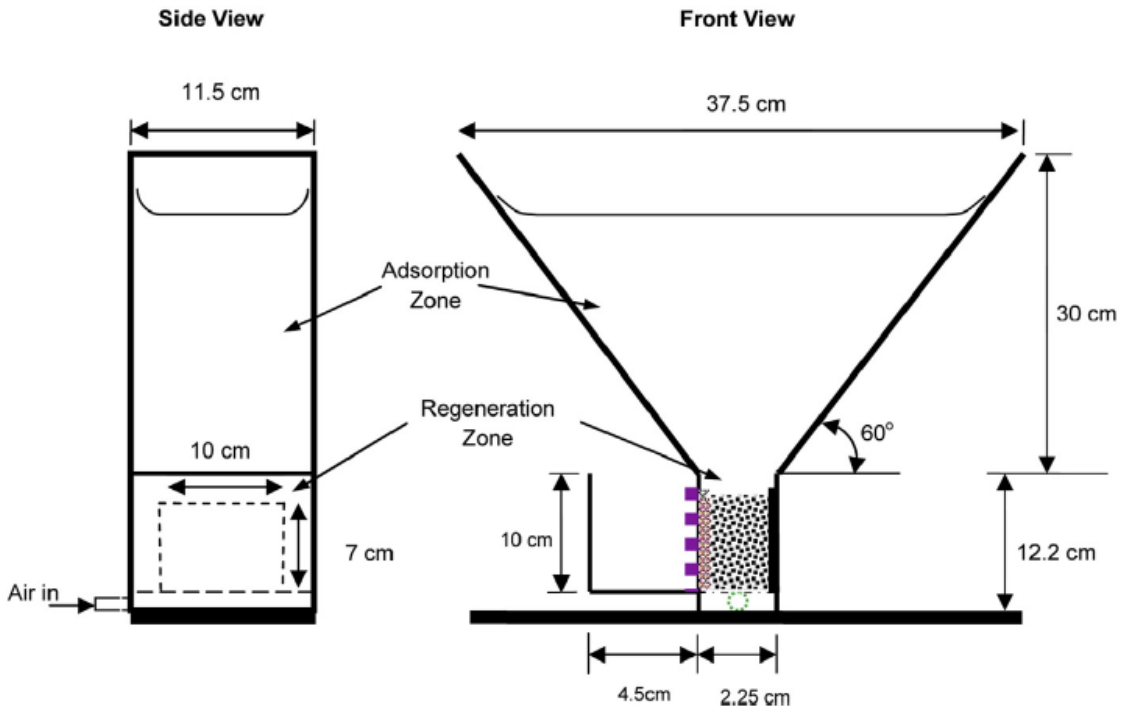

Fig. 1. Schematic diagram of the mini-sequential batch reactor (Hussain et al., 2012).

During the adsorption of P. aeruginosa onto the GIC adsorbent, a mixing time of 30 min was applied [18]. Regeneration of the GIC adsorbent loaded with P. aeruginosa was carried out at 30 mA cm_2 for 20 min in accordance with the conditions that were optimised for E. coli at neutral pH [18]. The results indicate that a high removal of P. aeruginosa was achieved with the GIC adsorbent with ca. 3.0 log10 reduction during the first adsorption cycle and even higher in the subsequent adsorption cycles (Fig. 2a).

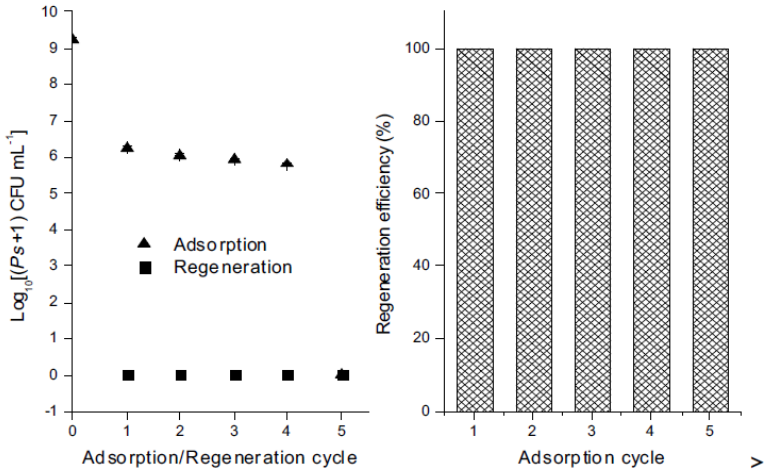

Fig. 2. Electrochemical regeneration of *P. aeruginosa* loaded GIC adsorbent in phosphate buffer (pH 7.0) with an initial concentration of 1.8×10^9 CFU mL^{-1}, solution volume: 300 mL; adsorbent mass: 60 g; regeneration current: 0.6 A corresponding to a current density of 30 mA cm^{-2}, regeneration time: 20 min at 30 mA cm^{-2}, anode current feeder (20 cm^2). The catholyte was 0.3% NaCl acidified with HCl (a) adsorption and regeneration cycles; (b) regeneration efficiency, where cycle 1 was carried out with fresh adsorbent. Each data point in figure (a) represents the average of five samples and the error bars indicate standard errors calculated from the standard deviation. In order to show the zero point on the logarithmic plot 1 CFU mL^{-1} was added to each data point on the vertical axis where Ps denotes the concentration of *P. aeruginosa*.

In addition, the GIC adsorbent was regenerated effectively with 100% regeneration on each adsorption cycle as shown in Fig. 2b. No P. aeruginosa was detected in the water after the regeneration cycles indicating that electrochemical disinfection of P. aeruginosa in solution was effective under the conditions used in this experiment (Fig. 2a). Thus, it can be concluded that a high concentration of P. aeruginosa in water can be disinfected during adsorption and electrochemical regeneration using the GIC adsorbents. Staphylococcus aureus Water contaminated with S. aureus was prepared in phosphate buffer deionised water to keep the pH of the water at 7 throughout the course of the experiment. Normally, the treated water in the mini-SBR after each cycle of regeneration is replaced with a fresh

bacterial suspension of same volume and concentration for the next adsorption cycle (see section Batch adsorption and electro- chemical regeneration). Keeping in view handling small volumes of pathogenic S. aureus, the behaviour when the same suspension of S. aureus was treated over several cycles was investigated by not siphoning off the treated water at the end of each regeneration. In all other respects the procedure was as described for batch adsorption and electrochemical regeneration in section Batch adsorption and electrochemical regeneration. Thus, a number of adsorption and regeneration cycles were carried out even at relatively low current density (10 mA cm_2) for S. aureus in water at pH 7.0. Prior to this experiment, studies of S. aureus adsorption onto the GIC adsorbent were carried out to evaluate the time required to achieve equilibrium and the adsorptive capacity according to a similar procedure to that described for E. coli [18]. The kinetic study indicated that the equilibrium time was around 30 min (data not shown), and the same adsorption time was used during adsorption cycles in the mini-SBR. The results suggest that a high concentration

of S. aureus was removed by adsorption and electrochemical regeneration with almost no detectable S. aureus after the second regeneration cycle in the mini-SBR as shown in Fig. 3. This also suggests that the process is quite effective in removing S. aureus present in water with a significantly higher reduction in the number of bacterial cells (ca. 9-log10 reduction) by treating a specific volume of water containing a high concentration of S. aureus over a number of cycles. Legionella pneumophila Test trials of adsorption and electrochemical regeneration with GIC adsorbent to disinfect L. pneumophila in water were carried out at University of Brighton in collaboration with the School of Pharmacy and Bimolecular Sciences. Initially, few drops of safranin were added to a mixture of GIC particles and water containing L. pneumophila in a small test tube. The contents of the test tube were thoroughly shaken and then centrifuged at 10,000 _ g for 10 s. Afterwards, the supernatant was removed from the test tube and a few drops of deionised water were added followed by centrifugation with the same conditions. Finally, a few particles of GIC adsorbent were removed from the test tube and were placed on a glass side. These particles were then viewed through the microscope (Olympus, BH-2) at 100_ magnification with the aim to investigate whether coloured species (L. pneumophila) were attached to the GIC particles. Disinfection of water containing L. pneumophila by adsorption and electrochemical regeneration was carried out according to the procedure described above for S. aureus. A same batch of L. pneumophila was treated over several cycles of adsorptions and regenerations by not siphoning off the treated water at the end of each regeneration.

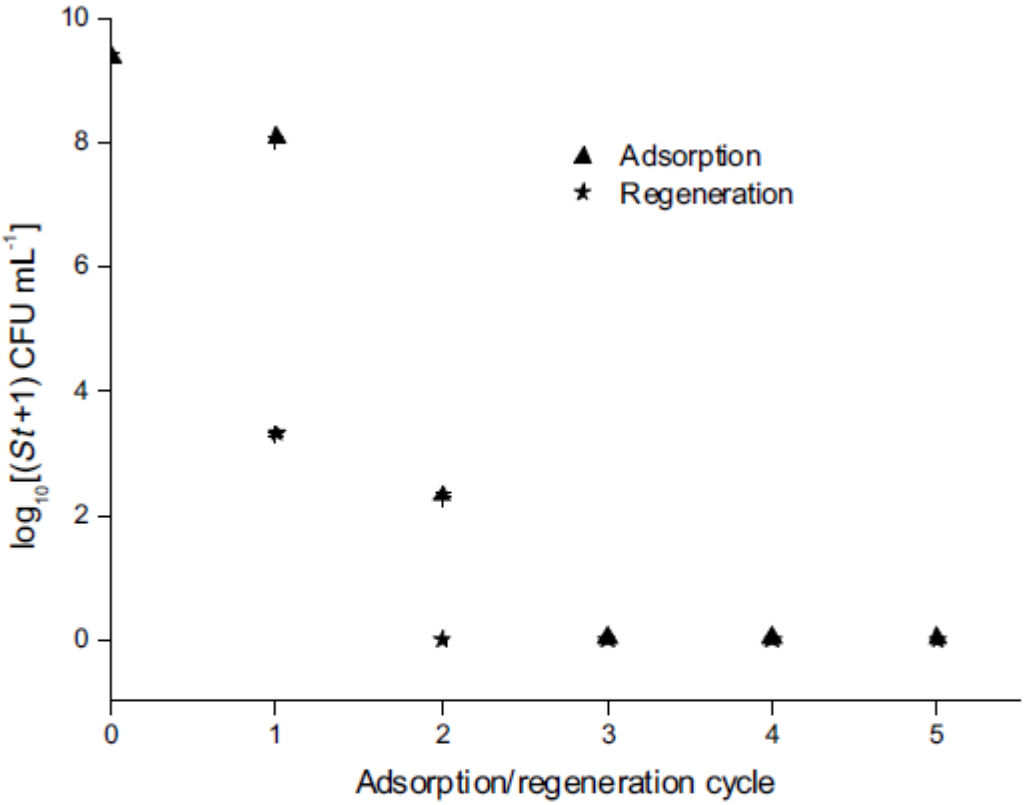

Fig. 3. Multiple adsorption and regeneration cycles with the same initial 1 L of 2.4×10^9 CFU mL^{-1} *S. aureus* (prepared in phosphate buffer pH 7.0) with 150 g of GIC adsorbent in the mini-SBR at 10 mA cm^{-2}, regeneration current: 0.5 A applied for 20 min (anode current feeder, 50 cm^2). The catholyte was 0.3% NaCl acidified with HCl. Each data point represents the average of five samples and the error bars indicate standard errors calculated from the standard deviation. In order to show the zero point on the logarithmic plot 1 CFU mL^{-1} was added to each data point on the vertical axis where St denotes the concentration of *S. aureus*.

It has already been seen that 30 min was required to achieve equilibrium during adsorption of E. coli, P. aeruginosa and S. aureus onto GIC adsorbent. Thus, the same time (30 min) was also applied for the adsorption of L. pneumophila onto GIC adsorbent in the mini-SBR. The electrochemical regeneration of E. coli loaded GIC adsorbent was effective at 10 mA cm_2 when no phosphate buffer was added into water to maintain a neutral pH [18]. However, electrochemical regeneration of GIC adsorbent loaded with L. pneumophila (when no phosphate buffer was added) was performed at 20 mA cm_2 because this bacterium has proved to be a refractory pathogen which is capable of resisting disinfection even after repeated cycles of chlorination [33]. The results indicated that a high concentration (2.6 _ 107 CFU mL_1) of L. pneumophila was removed by adsorption and electrochemical regeneration with no L. pneumophila cell detected in the water after the first regeneration cycle as shown in Fig. 4. About 95% of the L. pneumophila present in the water was removed during the first adsorption onto the GIC adsorbent which was effectively regenerated in the subsequent regeneration cycle (Fig. 4).

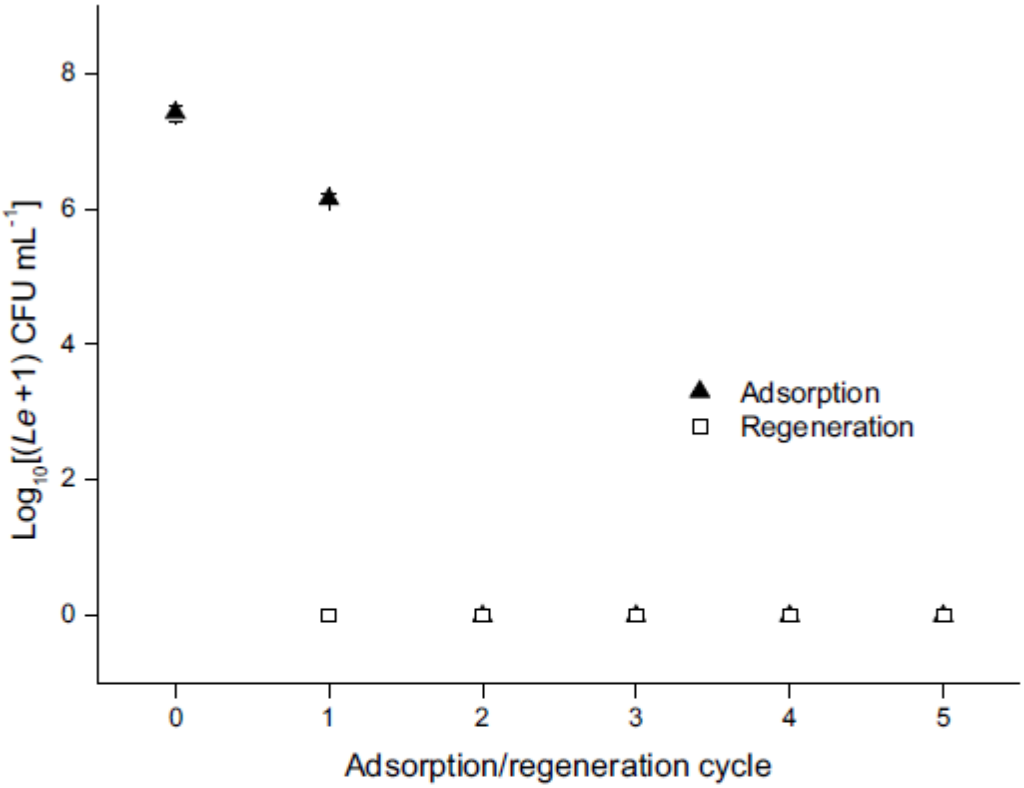

Fig. 4. Multiple adsorption and regeneration cycles with the same initial 1 L of 2.6×10^7 CFU mL^{-1} *L. pneumophila* with 150 g of GIC adsorbent in the mini-SBR at 20 mA cm^{-2}, regeneration current: 1.0 A applied for 20 min (anode current feeder, 50 cm^2). The catholyte was 0.3% NaCl acidified with HCl. Each data point represents the average of five samples and the error bars indicate standard errors calculated from the standard deviation. In order to show the zero point on the logarithmic plot 1 CFU mL^{-1} was added to each data point on the vertical axis where Le denotes the concentration of *L. pneumophila*.

Thus, the process of adsorption and electrochemical regeneration of GIC adsorbents was found to be efficient in removing L. pneumophila present in water with a ca. 7.5-log10 reduction in the number of bacterial cells under these conditions. Further trials will be required to assess the regeneration of L. pneumophila loaded adsorbent over a number of adsorption and regeneration cycles when after every regeneration the treated water is replaced with a fresh bacterial suspension of same volume and concentration for the next adsorption cycle. The adsorption of L. pneumophila on the GIC adsorbent was confirmed from the images of the GIC particles taken under a microscope (Olympus, BH-2) at 100_ magnifications after staining with safranin according to the procedure as described above. Attachment of the stained bacteria on the GIC particle indicates the adsorption of L. pneumophila onto the adsorbent (Fig. 5). In addition, the L. pneumophila were observed to be attached with the adsorbent particles in the form of clusters, particularly on the edges of the GIC particles. Some of the samples of the GIC adsorbent after electrochemical regeneration for 20 min at 20 mA cm_2 were also stained with safranin. The images for the regenerated

sample indicated that the coloured species initially adhering to the adsorbent particles had completely disappeared (Fig. 5).

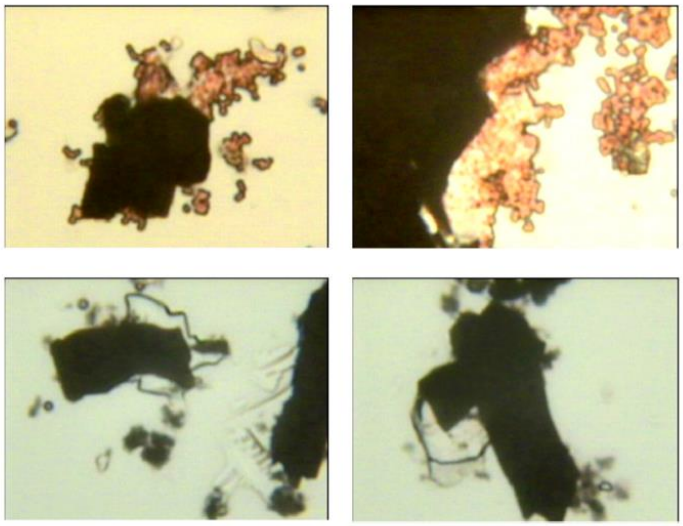

Fig. 5. Microscope images (at 100× magnification) of GIC particles after staining with safranin after adsorption (upper) and electrochemical regeneration (lower) in the mini-SBR, Regeneration conditions: 1.0 A was applied for 20 min (20 mA cm^{-2}).

Fungi

Aspergillus awamori

The kinetic study for the adsorption of A. awamori onto GIC adsorbent suggests that 30 min was required to achieve equilibrium (Fig. 6a). These results are similar to the adsorption of bacteria including E. coli, P. aeruginosa and S. aureus. The results for sequential adsorption and electrochemical regeneration in the mini-SBR show the removal of fungal spores during these processes (Fig. 6b) leading to regeneration efficiency above 96% for each adsorption cycle. The data suggests that during the electrochemical process in the mini-SBR, the fungal spores were removed completely from the solution due to indirect oxidation (Fig. 6b). The adsorption of fungal spores onto the GIC adsorbent along with its electrochemical regeneration was further confirmed from the SEM images as shown in Fig. 7. The adsorption of fungal spores on the GIC adsorbent shows clustering of the spores on the surface, presumably due to spore to spore interactions of the spores on the surface. In addition, some of the spores were observed to adsorb one above the other (i.e. multilayer adsorption). A high population of spores was observed on the relatively rough surface of the GIC particle. The SEM image taken after regeneration confirms the complete destruction of the spores onto the GIC surfaces.

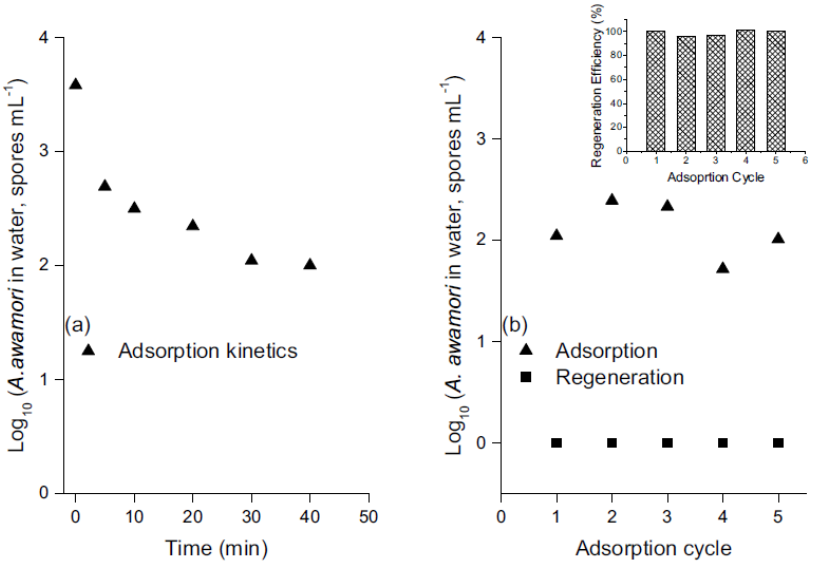

Fig. 6. Removal of *A. awamori* from water by adsorption and electrochemical regeneration using the GIC adsorbent, (a) adsorption kinetics: initial concentration 3800 spores mL^{-1}; GIC adsorbent dose 60 g in 300 mL (b) sequential adsorption and electrochemical regeneration in the mini-SBR, regeneration was carried out at 0.4 A for 20 min (20 mA cm^{-2}, anode current feeder: 20 cm^2). In order to show the zero point on the logarithmic plot 1 CFU mL^{-1} was added to each data point on the vertical axis where As denotes the concentration of *A. awamori*.

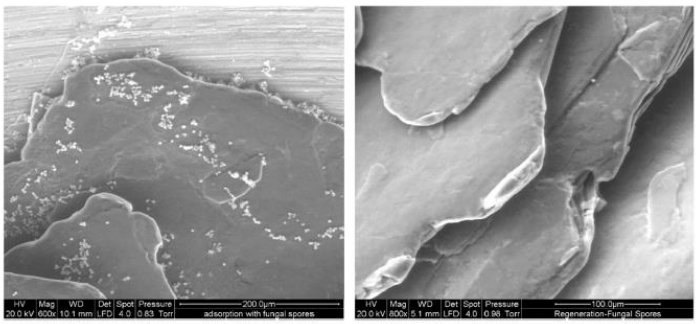

Fig. 7. Scanning electron micrographs of GIC adsorbent after adsorption of *A. awamori* (left) at 600× magnification and after electrochemical regeneration (right) at 800× magnificaition. Regeneration was carried out at 0.4 A for 20 min (20 mA cm^{-2}).

Yeast

Saccharomyces cerevisiae

A series of adsorption and electrochemical regeneration cycles were carried out according to the procedure as described in section Batch adsorption and electrochemical regeneration. The results suggest the removal of S. cerevisiae from water by adsorption and its subsequent electrochemical regeneration in a way that reasonably high regeneration efficiency is achieved after each adsorption cycle (Fig. 8b). During the electrochemical regeneration of the GIC adsorbent, the oxidants produced were effective in disinfecting the S. cerevisiae in water leading to complete disinfection after each regeneration cycle, as shown in Fig. 8a. In addition to the removal of yeast from water, these findings may also have implications for the treatment of beer. In the beer industry, the clarification of beer is usually followed by pasteurisation so as to ensure the microbiological stability and the conservation of beer. Conventional heat treatment requires water loops to heat and cool the product and also induces an additional water and energetic consumption. Sterile filtration is another treatment but it faces several challenges [34]. One alternative to pasteurisation step could be the use of adsorption and electro-chemical regeneration to sterilise the beer before conditioning. However, this application is beyond the scope of present study.

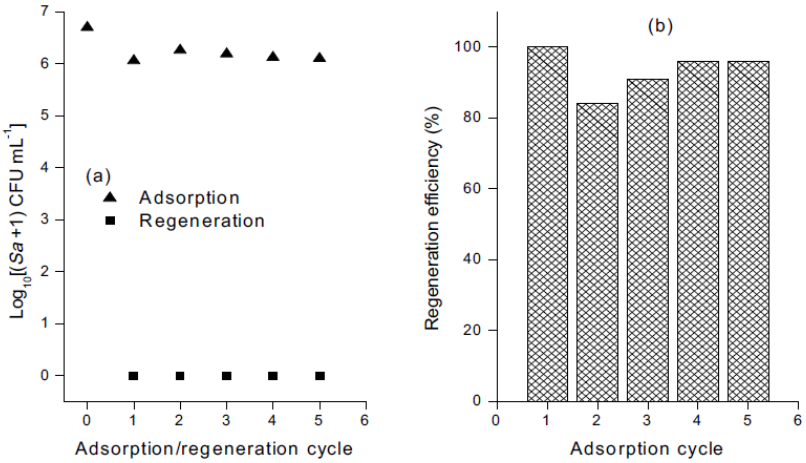

Fig. 8. Multiple adsorption and electrochemical regeneration cycles with *S. cerevisiae* in water in the mini-SBR, initial concentration: 5×10^6 CFU mL^{-1}, GIC adsorbent dose 60 g in 300 mL, regeneration was carried out at 0.4 A for 20 min (20 mA cm^{-2}). In order to show the zero point on the logarithmic plot 1 CFU mL^{-1} was added to each data point on the vertical axis where Sa denotes the concentration of *S. cerevisiae*.

Rhodosporidium turoloides

A series of adsorption and electrochemical regeneration cycles were performed according to the procedure as described in section Batch adsorption and electrochemical regeneration. The results indicated that ca. 96% of R. turoloides present in water were removed during the first adsorption and complete disinfection of the yeast was achieved during the subsequent regeneration. Regeneration efficiencies of above 100% were obtained for all the adsorption cycles (Fig. 9). Comparison with the results for S. cerevisiae shows that after second adsorption in case of R. turoloides not even a single colony forming unit was detected. This suggests that electrochemically generated oxidants entrapped within the adsorbent bed were more effective against R. turoloides than S. cerevisiae. The adsorption of R. turoloides was further confirmed from the SEM image as shown in Fig. 10. Clusters of R. turoloides were observed to be adsorbed on the edges of the GIC particles, suggesting that there were more yeast cells on the edges than the planar surfaces. Further work should be carried out to see if the yeast cells were disrupted during electrochemical regeneration of the GIC adsorbent loaded with R. turoloides.

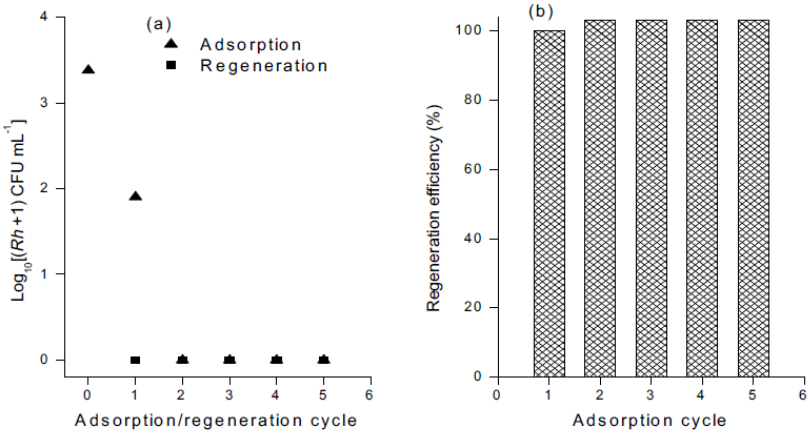

Fig. 9. Multiple adsorption and electrochemical regeneration cycles with *R. turoloides* in water in the mini-SBR, initial concentration: 2400 CFU mL^{-1}, GIC adsorbent dose 60 g in 300 mL, regeneration was carried out at 0.4 A for 20 min (20 mA cm^{-2}). In order to show the zero point on the logarithmic plot 1 CFU mL^{-1} was added to each data point on the vertical axis where Rh denotes the concentration of *R. turoloides*.

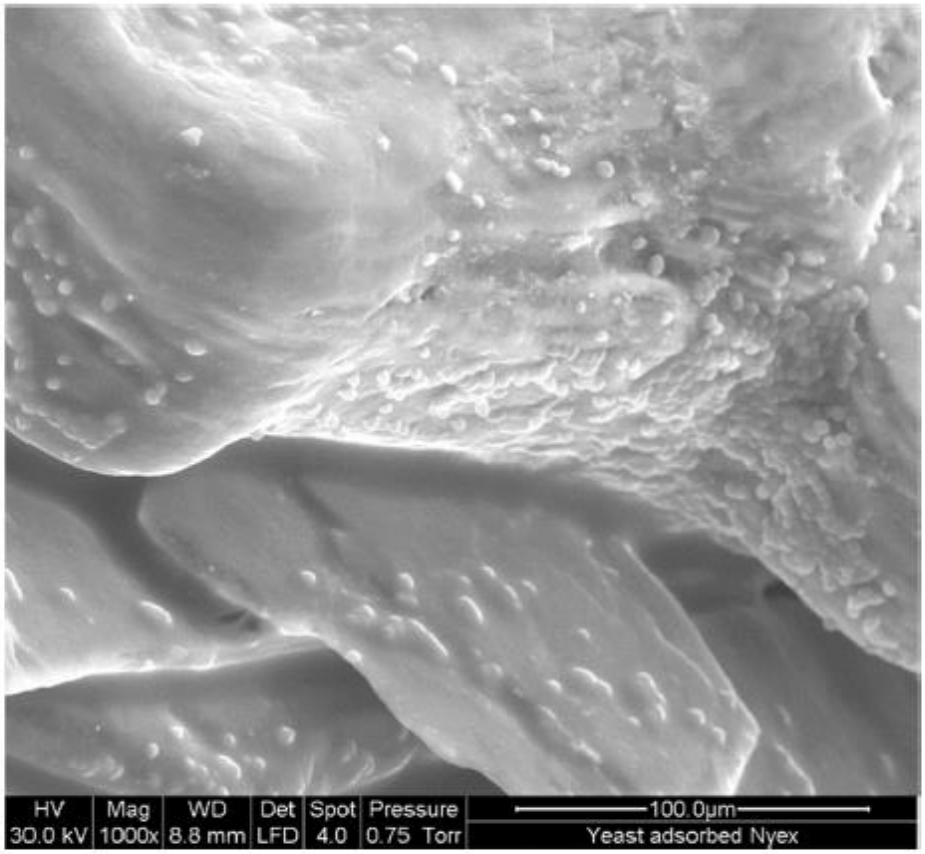

Fig. 10. Scanning electron micrograph of the GIC adsorbent with *R. turoloides* attached to its surface at a magnification of 1000×.

Protozoa

Cryptosporidium parvum

In order to investigate whether adsorption with electrochemical regeneration using the GIC adsorbents can be applied to disinfect protozoan organisms effectively, C. parvum was selected as a model species. The water containing oocycts of C. parvum was transferred to the mini-SBR in which 100 g of GIC adsorbent was added. A series of adsorption and electrochemical regeneration cycles were carried out, treating the same batch of water on each cycle. In all other respects, the procedure was as described for batch adsorption and electrochemical regeneration in the mini- SBR (see Section section Batch adsorption and electrochemical regeneration). Adsorption was carried out for 30 min and electrochemical regeneration was performed at 20 mA cm_2 for 20 min. After adsorption and regeneration, water samples with fines of GIC particles were collected. A small drop of a sample was placed on a microscope slide and viewed under a microscope which was connected to a camera and computer. Some of the samples were also stained using DAPI (Life TechnologiesTM) on the microscope slide and viewed under the microscope. C. parvum oocysts are spherical and of 4–6 mm in diameter with a characteristic shape and dark granules (Fig. 11). During adsorption, some of the fines of GIC particles were observed to attach to the C. parvum cell as shown in Fig.12. Based on SEM images (not shown), the C. parvum was not found to adsorb onto the large GIC flakes. The cells of C. parvum appeared to be covered with a thick cell wall (Fig. 12). However, during electrochemical regeneration, some of the images taken under microscope indicated the destruction or thinning of the cell membrane as shown in Fig. 13. C. parvum is known to be a refractory organism resistant to

chlorination and other disinfec-tants. In the cathode compartment, 0.3% NaCl aqueous solution was used as electrolyte and therefore due to the formation of free chlorine in the anode compartment containing the GIC adsorbent, Fig. 12. Images showing fines of GIC adsorbent attached to a C. parvum cell when viewed under a microscope.

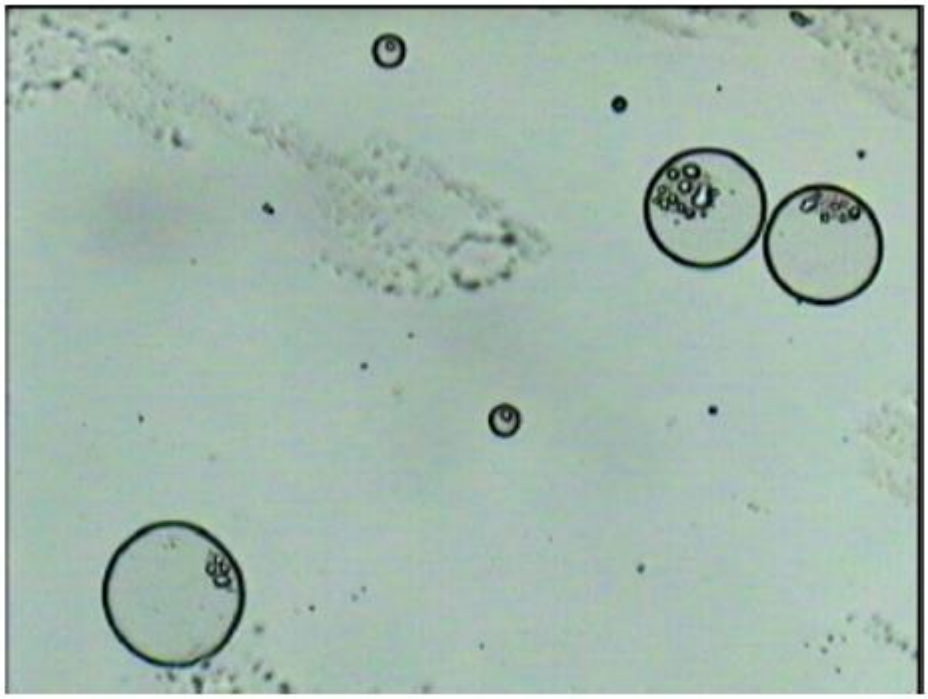

Fig. 11. *Cryptosporidium parvum* in water viewed under microscope at magnification 4.7 (BioMed, Leitz).

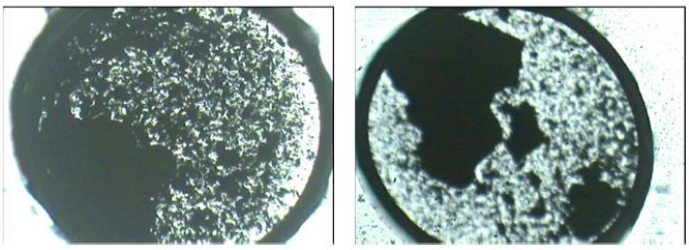

Fig. 12. Images showing fines of GIC adsorbent attached to a *C. parvum* cell when viewed under a microscope.

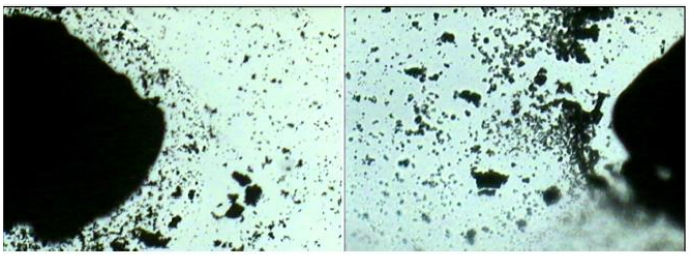

Fig. 13. Images taken during electrochemical regeneration of the GIC adsorbent for *C. parvum* under a microscope showing disintegration of the outer membrane of *C. parvum*.

electrochlorination is one of the most important factors responsible for disinfecting microorganisms in the mini-SBR [18]. As stated above, C. parvum are resistant to chlorination and therefore could not be disinfected. These were the preliminary trials that

suggest that changing the catholyte to a chloride free solution and applying relatively high current densities may disinfect pathogenic C. parvum. In addition, a protocol for determining the number of C. parvum cells after adsorption and electrochemical regeneration needs to be developed. In this context, the GIC particles after adsorption and regeneration were stained with DAPI (40,6- diamidino-2-phenylindole) to clearly visualise the change in morphology of the C. parvum during these processes but it was not successful due to the formation of DAPI needles on the microscope slide as shown in Fig. 14. All of these issues will be the subject of future work.

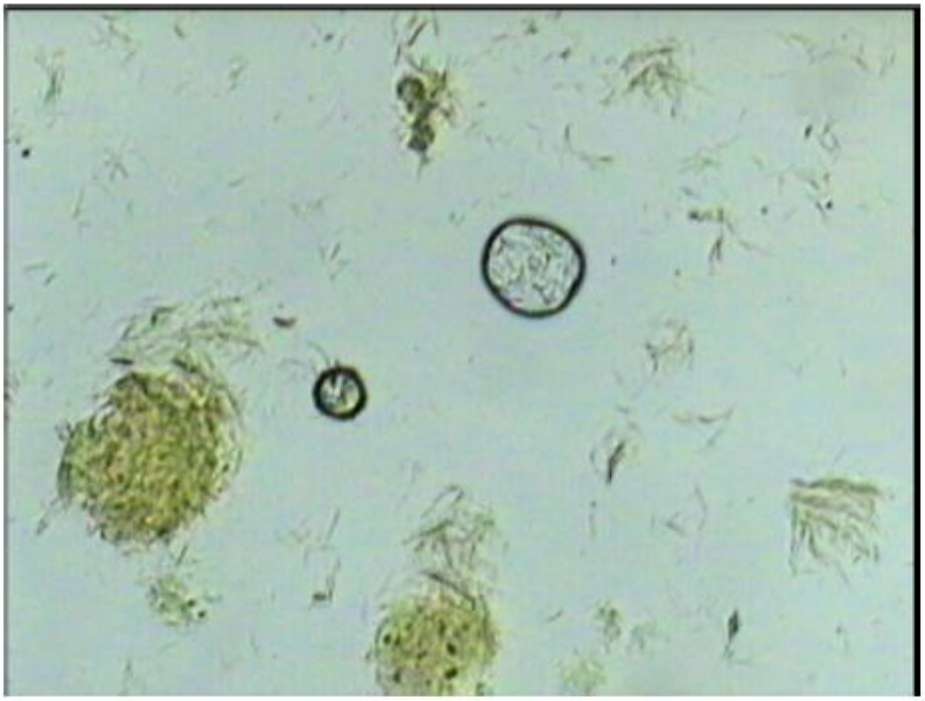

Fig. 14. Image of *C. parvum* in water stained with DAPI showing the presence of needle like contaminations.

Conclusions

The process of adsorption and electrochemical regeneration using the GIC adsorbents was found to be effective in removing a number of bacteria, fungi and yeasts. The bacteria species studied were P. aeruginosa, S. aureus and L. pneumophila. The suitability of the process was also evaluated for the fungal species A. awamori. In addition, the process was also found to be effective in disinfecting yeasts including S. cerevisiae and R. turoloides. Adsorption and electrochemical regeneration for some of these species were also confirmed from SEM images. On the other hand, disinfection of C. parvum by adsorption and electrochemical regeneration using the GIC adsorbent was not demonstrated successfully. However, a preliminary investigation regarding C. parvum suggests that using a chloride free solution in the cathode compartment and a relatively high current density could be effective.

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