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Retention of *E. coli* on Natural Pozzolan Beds

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Abstract: Micro organisms are wide spread in nature and when found in drinking water are known to be major precursors of many diseases. Their removal can be achieved through filtration in packed columns, thus reducing the amount of chemicals used for the disinfection of drinking water. Pozzolan from Djoungo (Cameroon) was tested as a potential filtration media for removing *E. coli*, the indicator of faecal contamination in ground and surface waters. Filtration experiments were carried out at room temperature (24±2°C) and microbial concentration was determined by the spread plate technique. Filtration tests were performed on 2 granular size media (450 and 900 μm mean size) at constant velocity (0.22 m h^{-1}), at different pH (5-9) and ionic strengths (10^{-4}-10^{-2} M). The aluminium sulphate concentration of 2.5 mg L^{-1} was used while the microbial concentration was fixed at 1.46×10^{2} cells mL^{-1}. Results showed that the best microbial retention was obtained at pH 5 and at a high ionic strength of 10^{-2} M KCl with coagulant dose (2.5 mg L^{-1}). The 450 μm mean diameter media presented a better efficiency compared to the coarse one.

Key words: *E. coli*, pozzolan, deep bed filtration, aluminium sulphate, water treatment, diseases

INTRODUCTION

Ground water is used as main source of drinking water in towns and rural area all over the world. Its turbidity level (<5 NTU), low salinity (<20 mg L^{-1}) and low amount of dissolved heavy metals in some areas allow it to be good for drinking purposes. Historically, ground water was assumed free from pathogenic microorganisms. Recent surveys indicate that a significant fraction of ground water supplies and surface water are a major source of waterborne diseases (Ndi, 2010). Many ground water sources in rural areas in Cameroon with clear natural springs have microorganism concentration exceeding WHO limits (Ndi, 2010). Their presence in water is a major threat to humanity. Micro organisms in water may result from agricultural practices like fertilization, defecation in water or disposal of human or animal waste in water and urban ills like careless disposal of house hold wastes.

These micro organisms are the major causes of diseases such as cholera, diarrhea and typhoid. Not only are these diseases expensive to treat but they are as well deadly if not diagnosed and treated on time. As the saying goes prevention is better than cure, it is better to take in potable water than curing or treating diseases caused by unsafe water. Potable water must be free from micro organisms in addition to its being tasteless, colourless and odourless. The contamination of water by micro organisms is generally expressed by the presence of *E. coli*; the indicator of faecal contamination. In remote rural areas where ground water and natural springs with elevated micro organisms' levels are the only easily accessible water sources their content should be reduced to acceptable levels.

Many methods are used to remove micro organisms from water. In water treatment plants, coagulation, sedimentation, filtration and disinfection steps help to reduce microbial charge as well as other contaminants in water. The last step is the most efficient in the reduction of microorganisms but need the use of high amounts of chemicals (Rooklidge *et al*., 2002). The efficiency of the disinfection step can be increased by increasing the efficiency of the previous steps (coagulation,

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sedimentation and filtration). Granular filtration has been found to be very effective for the retention of protozoan parasites such as *Giardia* and *Cryptosporidium*, difficult to eliminate during disinfection steps (Stevenson, 2003). As it is the last step of elimination of suspended particles in classical water treatment units, it should also be able to reduce considerably the amount of microorganisms from water. Many studies have shown that microorganisms could be attached on granular particles under favourable or unfavourable conditions, related to the surface chemistry of the grain particle and the bio colloid (Hann and O'Melia, 2004). Therefore coating sand with aluminium or ferric oxidesexhibited higher retention efficiency than natural sand (Bolster, 2001). Unlike coated sand, pozzolan, a natural volcanic material contains aluminium and ferric oxides on their surfaces (Messi *et al*., 1994; Ndi *et al*., 2008). It has been found to be more efficient than granular sand in the removal of clay colloidal particles (Ndi *et al*., 2006) and in this study its efficiency is evaluated on *E. coli* (a biocolloid) under various physicochemical conditions (pH, ionic strength, aluminum sulphate concentration and collector size).

MATERIALS AND METHODS

Biological material: Lyophilized *E. coli* of the k_{12} batch was obtained from the Microbiology laboratory of IUT-ENSAI. A pintchof lyophilised *E. coli* K12 strain was put in a sterilised test tube containing 1 mL of physiological solution and agitated using an electrical agitator until the bacteria got completely dispersed in the solution. About 0.1 mL of the *E. coli* strain was then inoculated on pre-prepared EMB (Eosine Methylene Blue) culture medium. After 24 h of incubation in an incubator at 37°C, growth of colonies was observed. An *E. coli* colony was obtained from the EMB medium and cultured in 150 mL of TSB (Tryticase Soya Broth) and incubated for 24 h in an incubator at 37°C. The medium became cloudier than before, indicating that growth of *E. coli* had occurred. The bacteria suspension in the TSB medium was then centrifuged in a Hereuse centrifuge at a rate of 6000 rpm and rinsed 3 times. This was to purify the bacteria strain by separating the bacteria from the TSB medium.

The pure bacteria strain was then suspended in physiological solution to avoid cell hemolysis and to maintain the physiological state of the cells. The bacteria in the suspension were then kept in a Memmert refrigirator at 4-10°C to avoid aging of the cells and to minimize cell multiplication. All other reagents used in the present study were of analytical grade.

Granular material: Granular material was prepared from pozzolan excavated from Djoungo in the Littoral region of Cameroon. The material was crushed, sieved and washed several times to obtain a final turbidity level <0.5 NTU. The resulting material had three granular sizes: 400-500, 630-800 and 800-1000 μm. It was dried at 105°C in an oven (Heraeus, Germany), allowed to cool down in adesiccator at room temperature and sealed in sterile plastic bags. It was then used without further purification. Recent researchers on this material using SEM/microprobe, revealed the presence of different oxides mainly iron oxides corresponding to goethite and hematite and aluminium oxide (gibbsite) and titanium dioxide.

The granular pozzolans of Djoungo has an isoelectric point (iep) located around pH 3, with a negative surface charge within the range of experiments (<-30 mV) (Stevenson, 2003).

Filtration experiments: The filtration plant consists of two supply tanks, stirred with a magnetic stirrer as shown in Fig. 1. A membrane pump allows the transfer of the fluid through the glass filtration column. The internal diameter of the column is 24 mm. Prior to use, tubes and column were cleaned thoroughly with tap water and soap. They were then disinfected with chlorinated water at 25% dilution and thorough rinsing with abundant distilled sterilised water followed.

After packing column with the granular material, distilled water, of fixed pH and ionic strength, was used to flush the bed for >30 min in an upflow mode. Before the retention experiments, the suspensions are coagulated by gently introducing with a syringe, the exact amount of commercial aluminumsulphate (1 g L^{-1}) in the suspension, maintained under agitation. This mixing would prevent sedimentation in the source water tank. The coagulated suspension was then introduced into the column in an upward flow mode. At the exit of the column the filtrate was collected as a function of time and analyzed by cell enumeration.

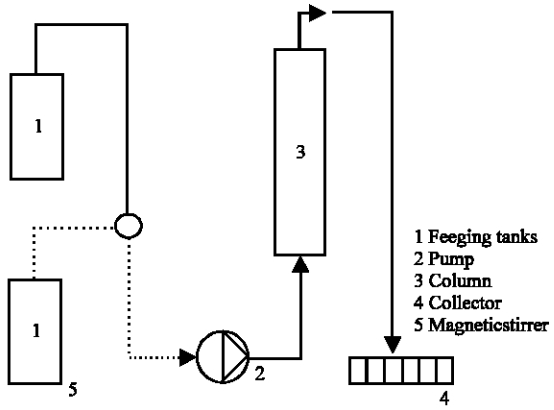

Fig. 1: Schematic diagram of apparatus used

Cell enumeration: Suspended *E. coli* in the filtrate obtained was enumerated by spread plate technique. Appropriate sample dilutions (for feed of initial bacterial concentration of 1.46×10^{2} cells mL^{-1} of feed) were obtained by serial dilutions in physiological solution. The plating media were MacConkey and Eosin Methylene Blue Agar.

For feed with an initial bacteria concentration of 1.46×10^{2} cells mL^{-1}, since less cells were suspected in the filtrate, cell enumeration proceeded by membrane filtration using Whatman sterile membrane filters or nylon plain membrane filters with pore size equal to 0.45 μm. Membrane filtration was followed by inoculation on MacConkey or Eosin Methylene Blue Agar in Petri dishes. They were further incubated at 37°C for 24-48 h which was time enough to allow for growth.

RESULTS AND DISCUSSION

Effect of pH: The results of the influence of pH on the retention of *E. coli* on pozzolan grains are shown in Fig. 2. Increasing the pH did not change notably the overall amount of *E. coli* retained in the pozzolan bed at low ionic strength (10^{-4} M Kcl) and at different pH (5, 7 and 9). The log (C)/log (C_0) is within the range of 0.6-0.8 (<1) and is almost constant with the reduced volume of filtrate collected. As shown, the retention is observed within the first 5 min and does not change with the reduced volume of filtrate. As the ratio log (C)/log (C_0) is <1, it can be deduced that in these conditions, an amount of *E. coli* is retained in the bed.

With low ionic strength cell-cell and cell-collector interactions are reduced (Elimelech, 1991). So, cell aggregation in the suspension or in the bed is not the main mechanism involved (Hann and O'Melia, 2004). Increasing the pH from 5-9, should have change the surface charge on pozzolan grains and on *E. coli* cells. But as shown by the results changing the pH did not result in a drastic change in the overall amount of *E. coli* retained in the pozzolan bed. As the surface charge of pozzolan grains and *E. coli* at low ionic strength and at pH >5 is negative (<– 30 mV) (Hann and O'Melia, 2004), the interaction between those particles can be assumed to be unfavorable (Puls *et al*., 1993). In these conditions, the observed retention could be explained by the cell-grain interactions under unfavorable retention conditions which has been attributed to the surface charge heterogeneity of pozzolan and the roughness of the collector (Elimelech, 1991; Puls *et al*., 1993).

Effectof ionic strength: Figure 3 shows theinfluence of the ionic strength on the retention of *E. coli* at pH 5. No significant difference was observed at 10^{-4} and 10^{-3} M KCL while with ionic strength equal to 10^{-2}M KCL the amount of *E. coli* retained within the bed was greater than the previous cases. At 10^{-4} M KCl and 10^{-3} KCl, the ionic cloud around the particle is very large, compared to 10^{-2} M KCL, thus at high ionic strength collision efficiency of cell-cell and cell-collector increases as it reduces cell-celland cell-collector separation distance. This trend is coherent with what have been found by other on the influence of the ionic strength on the retention efficiency (Bai and Tien, 1999).

Effect of aluminium dose and cell concentration: Figure 4 shown the results for the retention of *E. coli* at different concentrations and different amount of aluminumsulphateused. Without any coagulant used, no significant retention was observed for both 146 and 1460 UI mL^{-1} amount of *E. coli* in the suspension. As shown here as earlier, without any coagulation, less amount of *E. coli* is retained in the bed while using aluminium

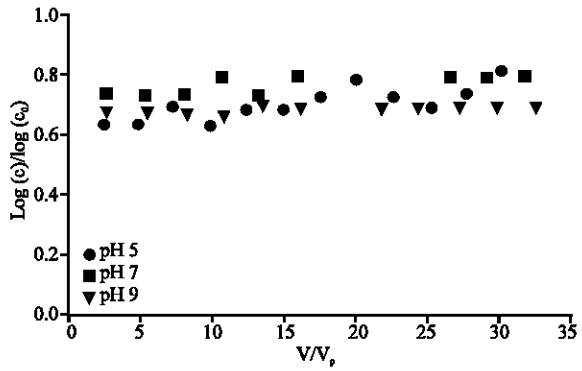

Fig. 2: Log (C)/Log (C_0) versus V/Vpat pH 5, 7, retention on granular pozzolan (400-500 μm), equivalent ionic strength 9, 10-4 M KCL

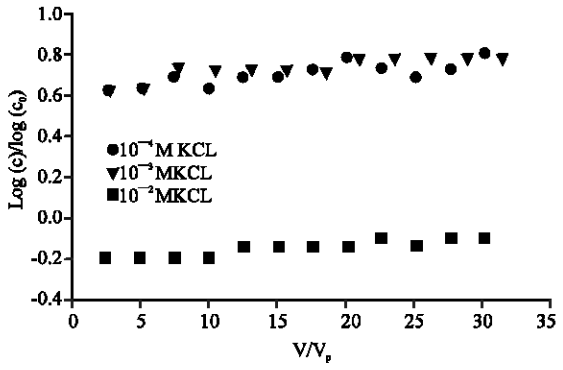

Fig. 3: Log (C)/Log (C_0) versus V/Vpat equivalent ionic strength of 10-4, 10-3 and 10-2 MKCL, retention on granular pozzolan (400-500 μm) at fixed pH 5

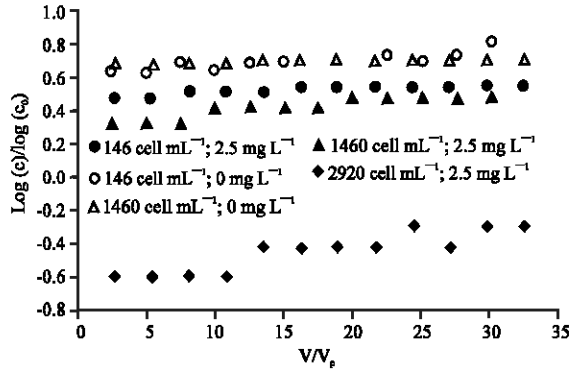

Fig. 4: Log (C)/Log (C_0) versus V/Vpat different aluminum dose

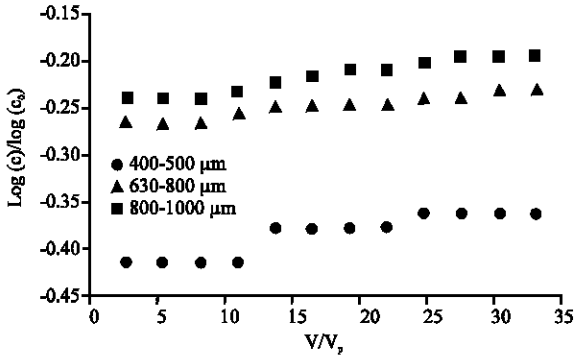

Fig. 5: Log (C)/Log(C0) versus V/Vpat different collector size

sulphate improved its retention. Increasing the amount of particles up to 2920 cell mL^{-1} using the same amount of coagulant as in the previous experiments gave better retention of *E. coli* but the retention mode was found to be less stable as the release of *E. coli* cells was observed with the reduced filtrate volume.

The amount of coagulant used (2.5 mg L^{-1}) corresponds to the highest concentration of aluminum sulphate which could be used for the removal of clay particles in a suspension of 60 NTU (Kretzschmar *et al*., 1994). Therefore, for the retention of a microorganisms found in a water of the same type, using this maximum concentration of aluminum sulphate could give us an indication on the capture mechanism involved.

The best retention was observed for the more concentrated suspension. This behavior could be explained by the concentration of the suspensions used. For dilute suspensionscollision efficiencies between particles is low, indicating that the aggregates generated from those diluted suspension would have low dimensions. For more concentrated solutions, as the coagulation is effective on those particles, the aggregates size would be greater than in the first case (Puls *et al*., 1993). Thus, cell-cell interactions where very pronounced at 2.5 mg L^{-1} of aluminum sulphate used. So, the retention of *E. coli* suspensions at low concentration will be less efficient than the retention of a more concentrated solution in pozzolan granular bed if aluminum sulphate is used as a coagulant.

Effect of media size: The influence of collector size is shown in Fig. 5. Retention experiments where carried out with aluminum sulfate (2.5 mg L^{-1}) at 10^{-3} M KCl, pH 5 and at various pozzolan grain sizes. As the size of the collector increases, the overall retention of *E. coli* cells in pozzolan beds decreases. So, compared with other collector size, greater retention was found with pozzolan grains of 400-500 µm. After 10 V/Vp, the released of *E. coli* cells in pozzolan bed (400-500 µm) is higher than in the other beds.

About 2 mechanisms may explain the capture observed in the bed. As the size of the collector size is reduced, the hydraulic radius is also reduced and the collision efficiency between cell-cell and cell-collector increases in the bed (Hann and O'Melia, 2004). Also, reducing the size of the particles increases the screening effect of the bed. So, coupling aggregation and screening effect of the bed would increase the amount of *E. coli* retained in the bed. In this condition, more cells may be retained in the bed and with hydraulic flow thry may be detached as found in this present case (Hann and O'Melia, 2004).

CONCLUSION

Retention of low charge of *E. coli* in water through granular pozzolan bedscan be achieved without using aluminum sulphate as a coagulant at different pH (Hann and O'Melia, 2004; Bolster, 2001; Messi *et al*., 1994; Ndi *et al*., 2006, 2008). Using aluminum sulfate increases the observed retention and has been found to be very effective on pozzolan granular size of 400-500 µm. Sousing pozzolan grains for filtration process may reduce the overall amount of chemicals use for disinfection.

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