Practical Paper

The retention of *Cryptosporidium* sp. oocysts at varying depths in slow sand filters: A pilot study

Léo Heller and Ludmila Ladeira Alves de Brito

ABSTRACT

The retention of protozoa cysts and oocysts at varying depths in slow sand filters is a subject still not completely understood. A better understanding may contribute to an improved assessment of the risk of infections in those populations that receive water treated by slow sand filtration and could also contribute to the improvement of the planning and design of slow sand filters. With these aims, this paper intends to communicate advances in the understanding of the retention of *Cryptosporidium* oocysts and microbiological indicators of water quality in the filter media. In the study, *Cryptosporidium* oocysts presented a similar behaviour for the higher filtration rate ($6\,m^3\,m^{-2}\cdot d$) in both flows investigated. For lower filtration rates ($3\,m^3\,m^{-2}\cdot d$), the penetration of these oocysts is higher in the downflow filter. In the upflow filter the retention is limited to the initial 0.45 m of the filter media. There was no accumulation of oocysts inside the filter media as the run progressed, suggesting the existence of some control mechanism. The *schmutzdecke* seems to play an effective role in the removal of protozoa only when it is well developed. A weak relationship was found between the *Cryptosporidium* oocysts and both water quality and treatment efficiency indicators.

Key words | *Cryptosporidium*, microbiological indicators, *schmutzdecke*, slow sand filtration, turbidity

Léo Heller (corresponding author)
Department of Sanitary and Environmental Engineering, School of Engineering,
Universidade Federal de Minas Gerais,
Brazil, Av. Contorno, 842/701 – 30.110-060,
Belo Horizonte,
Brazil
Tel.: 0055-31-3238-1958
Fax: 0055-31-3238-1879
E-mail: *heller@desa.ufmg.br*

Ludmila Ladeira Alves de Brito
IBAMA, Brazilian Institute of the Environment and Renewable Natural Resources,
Ministry of the Environment,
Brazil

INTRODUCTION

Although the slow sand filtration method of water treatment has been in use for over 170 years, a deeper understanding of its contaminant removal mechanisms is still required. This information is important particularly in regard to the removal of emerging pathogens, in order to obtain elements for the optimisation of this function of the process.

Several authors (Huisman 1982; Vargas 1999) have reported that, in microorganism removal by the filter, not only the natural screening acts, but there is also a combination of physical, chemical and, above all, microbiological factors, such as predation, competition and natural death. The current understanding of these mechanisms indicates a potentially satisfactory removal of *Giardia* cysts and *Cryptosporidium* oocysts. For this purpose, it is necessary to understand how the removal of these pathogens takes place spatially inside the filters. This could contribute to the understanding of the retention mechanisms and help to direct the project and operational decisions in order to improve the safety of the process.

Several studies have been performed for evaluation of the efficiency of slow sand filters in removing pathogens and their indicators, and high removal rates could be observed most of the time. For example, Fox *et al.* (1984) reported

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a consistent removal of 4 to 5 log of total coliforms in pilot-scale filters. Bellamy *et al.* (1985a), also studying pilot filters, found a removal rate higher than 99% for total and thermotolerant coliforms. A result confirmed by Murtha & Heller (2003), who found no *Escherichia coli* concentration values in the effluent of pilot filters in approximately 90% of the results. Poynter & Slade (1977) achieved a 99.997% virus removal. In addition to the high removal rates, it was determined that only 2% of the influent viruses remained viable on the sand layer (McConnel 1984).

Regarding protozoa, it seems that, consistently, the removal of *Cryptosporidium* by slow sand filters is lower than that of *Giardia*. By evaluating a full-scale plant, Fogel *et al.* (1993) found 93% of *Giardia* cysts removal and just 48% for *Cryptosporidium* oocysts removal. The low removal rates can be possibly attributed to the high uniformity coefficient of the media and to the low ambient temperatures (<1°C). Bellamy *et al.* (1985b) did not detect *Giardia* cysts in the filter effluent with the sand media already biologically mature, while the slow sand filter studied by Timms *et al.* (1995), operating at a filtration rate of $9.6\,m^3\,m^{-2}\cdot d$, achieved approximately 99.997% of *Cryptosporidium* oocysts removal. By testing pilot filters at rates of 3 and $6\,m^3\,m^{-2}\cdot d$, Vieira (2002) found 97.035% removal for cysts and no detection of oocysts.

The importance of the filter media maturation was shown in pilot research (Bellamy *et al.* 1985b), in which a filter in recent operation removed 0.82 logs of total coliforms and 1.7 logs of *Giardia*, while the same filter, after two weeks of operation, removed 4 logs of total coliforms and released no *Giardia* cyst into the effluent.

Some studies aimed at evaluating the in-depth removal of indicators, such as that performed by Murtha & Heller (2003), showed that the initial 0.30 m of the filter media would be responsible for almost the total removal of total coliforms and *E. coli*. Timms *et al.* (1995) observed that just the initial 2.5 cm of the sand media of the filter retained *Cryptosporidium* oocysts.

In fact, the few studies published showed different situations of filter media maturation, temperatures and filtration rates, among other aspects, thus raising difficulties in the generalization of results.

On the other hand, due to the difficulties and cost of laboratory analysis of protozoa, the identification of surrogates that enable a more rapid and responsive operational evaluation of their removal in the treatment systems becomes an important task, as well as the development of suitable monitoring plans. So, a better understanding of the filtration efficiency indicators and the effect of the treatment on them is a priority for the protection of human health. Physical indicators, such as turbidity and particle counts, and microbiological indicators have been proposed as surrogates for protozoa cysts and oocysts. The microbiological indicators include the traditional coliform group; anaerobic bacteria spores and *Clostridium perfringens*; aerobic bacteria spores and *Bacillus subtilis* and total heterotrophic bacteria counts.

The bacteria of the coliform group have been traditional indicators of faecal contamination, and there is a current trend to adopt either the thermotolerant sub-group or *E. coli* for this purpose (WHO 2004).

C. perfringens is a species of the sulphite-reducing clostridium group, forming spores that survive under anaerobic conditions. The clostridium spores survive in water for much longer periods than the bacteria of the coliform group, having been used for a long time as indicators of remote faecal contamination. Due to their resistance, their presence in the effluent of a water treatment plant may mean filtration deficiencies (Araujo *et al.* 2001; WHO 2004). However, Nieminski *et al.* (2000) showed that these spores, which are usually present in raw water in very low concentrations, could not be used as indicators of the efficiency of the treatment. Ferguson *et al.* (1996), studying several indicators of the presence of protozoa in surface waters, observed that the only microorganism that correlated with *Cryptosporidium* and *Giardia* was *C. perfringens,* while Nieminski *et al.* (2000) observed no correlation between these protozoa and either *C. perfringens* or the total anaerobic spore counts.

The aerobic bacteria spores have been recommended as indicators of the performance of the treatment in removing protozoa cysts and oocysts, since they are highly resistant to several environmental factors, temperature and chemical compounds used in water disinfection processes (Barbeau *et al.* 1999). Studies by Rice *et al.* (1996) and Nieminski *et al.* (2000) showed that the indigenous aerobic bacteria spores were not satisfactory to indicate the presence of protozoa in water sources but, associated with other indicators such as

turbidity and particle counts, they acted as efficient indicators of the quality of the treatment.

Heterotrophic bacteria are usually defined as indicators for evaluating the general content of bacteria in water. Nieminski *et al.* (2000) emphasized that, in spite of their high concentrations in raw water, these microorganisms are able to grow inside sand filters, thus restricting their use as indicators of the efficiency of the treatment process. However, Bellamy *et al.* (1985a) and Ellis & Aydin (1995), studying the behaviour of slow sand filters under several environmental and operational conditions, selected the total heterotrophic bacteria as indicators of the biological maturation of the filter media.

Another theme still incompletely understood refers to the fate of the upflow slow sand filters (USSF), especially on microorganisms removal. This treatment method was first used in Scotland, and has the potential advantage of its more simplified cleaning procedure, based on the bottom discharge of the retained solids, when compared with the conventional downflow slow sand filters (DSSF). Because of this, a shallower media can be used. In USSF, the support layer works as a pre-filter, potentially improving the performance of the process. Previous research carried out by our group had shown that USSF could show a much longer duration of the filter run, compared with DSSF submitted to the same raw water. However, a remaining unanswered issue is the effect of a large number of bottom discharges on the media characteristics. (Valencia 1981; Vieira 2002; Murtha & Heller 2003).

In view of this present knowledge, this study aims at contributing to an advanced understanding of the dynamics of the *Cryptosporidium* oocysts and microbiological indicators of water quality at different depths of the filter media of slow sand filters. Regarding the specific objectives, the intention of the research was to evaluate the dynamics of this protozoan under different filtration rates; to compare its behaviour in the upflow and downflow filtration; to verify the possible correlation between different microorganisms and the protozoa in the slow sand filtration; and to evaluate the participation of the downflow slow sand filtration *schmutzdecke* in removing oocysts.

MATERIALS AND METHODS

Experimental apparatus

The pilot plant is represented in Figure 1. It consists of activated carbon filters, a water preparation box, a pump for transfer from the preparation box to the feeding box, a feeding box, a peristaltic pump to feed the filters, a downflow slow sand filter (DSSF), an upflow slow sand filter (USSF) and piezometers.

Each filter consisted of two 0.20 m diameter (based on the studies of Lang *et al.* 1993), 1.50 m high overlapped flanged acrylic pipes. A perforated plate was fastened to the lower part of each filter to support the granular media, located at 0.10 m from the base. In order to minimise 'wall effects' and short-circuiting, several circumferential lines of silicone were fixed in both filters, along their walls.

The water influent in the downflow slow filter was located 0.30 m below the top of the filter (1.45 m above the filter media), and the effluent outlet was just below the perforated plate, in an inverted 'U' shape, aimed at maintaining a minimum water level of 0.45 m above the

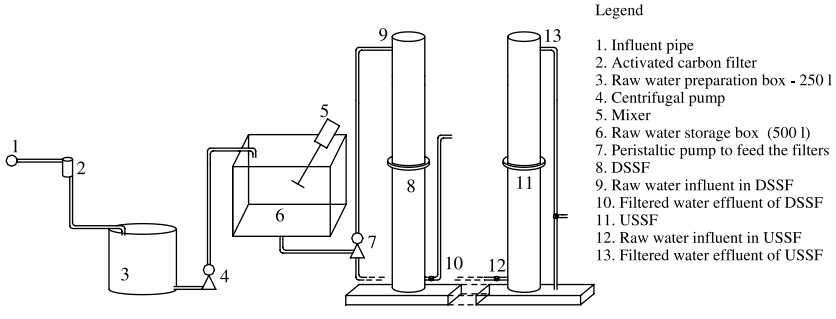

Figure 1 | Pilot plant scheme.

media. In the upflow slow filter, the inlet was located 0.10 m above the lower base of the filter and the outlet at 0.30 m below the top (1.45 m above the filter media).

The filter media in both filters consisted of sand, 0.75 m deep, effective size of 0.25 mm, with uniformity coefficient of 2.40, with a minimum diameter of 0.084 mm, and a maximum diameter of 1.00 mm. The supporting layer consisted of three gravel layers (depth: 0.10 m, grain size: 1.2–2.40 mm; depth: 0.10 m, grain size: 4.76–6.36 mm; depth: 0.20 m, grain size: 12.70–19.1 mm).

Synthetic water

The filters were fed with synthetic water suitable for treatment in a slow sand filtration process, with the following characteristics:

- turbidity: 15 ± 5 NTU
- true colour: 15 ± 5 CU
- pH: 6.5–7.5
- total coliforms: 10^5–10^6 MPN 100 ml^{-1}
- *Escherichia coli*: 10^3–10^4 MPN 100 ml^{-1}.

Besides these characteristics, doses of other microorganisms of interest and of the studied protozoa were added, as follows:

- *Clostridium perfringens* and anaerobic bacteria spores: 10^1–10^2 CFU 100 ml^{-1};
- Bacillus subtilis and aerobic bacteria spores: 10^4–10^5 CFU 100 ml^{-1};
- Cryptosporidium: 10^1–10^2 oocysts l^{-1};
- Giardia: 10^0–10^1 cysts l^{-1}.

The preparation of synthetic water used water from the public network, which had the chlorine removed by activated carbon filters and had the following substances added:

- bentonite, for generation of turbidity
- Paraguayan tea effusion, for production of the true colour
- 1N sulphuric acid, for pH correction
- an aliquot of sewage, which provided the microorganisms analysed (total coliforms, *E. coli*, *C. perfringens* and anaerobic bacteria spores, *B. subtilis* and aerobic bacteria spores, *Giardia* cysts)
- *Cryptosporidium* oocysts, produced in a newly born calf

Experimental outline and sampling

Each of the filters (DSSF and USSF) was evaluated under two different filtration rates (3 $m^3 m^{-2}$ ·d and 6 $m^3 m^{-2}$ ·d) generating four combinations. Two runs (duplicate) were performed for each of these situations, resulting in eight filter runs, four for each filter.

The analyses were made twice during each filter run, one at the beginning of the run, when the filter was not completely biologically mature, and another after the effluent had shown more stable quality, according to Table 1.

The basis of these times was chosen from previous experiments (Vieira 2002), which determined the duration of the filter runs and the phase when stable effluent began to be produced, with turbidity below 2 NTU, a value established using Brazilian standards.

The filter run was stopped for analysis by turning off the filter feeding system. The supernatant water in the filter was removed through the top of the unit, aided by a peristaltic pump, until it reached the top of the filter media. In the DSSF, the last 0.10 m of water on the layer was removed by using a siphon, in order to avoid disturbing the biofilm on the sand layer (*schmutzdecke*). After that, the filter was disassembled and the upper column was removed. The *schmutzdecke* (Figure 2) was initially scraped in the DSSF, and evaluated for the presence of the researched protozoa and microbiological indicators.

The sand media was divided into five 0.15 m thick layers (Figure 3), which were removed using a ladle, previously disinfected in a concentrated sodium hypochlorite solution. The material from every layer was subsequently washed in

Table 1 | Times of analysis in each run

Filtration	Downflow slow filter		Upflow slow filter	
Rate	1st analysis	2nd analysis	1st analysis	2nd analysis
3 $m^3 m^{-2}$ ·d	2nd day	8th day	2nd day	10th day
6 $m^3 m^{-2}$ ·d	2nd day	4th day	2nd day	4th day

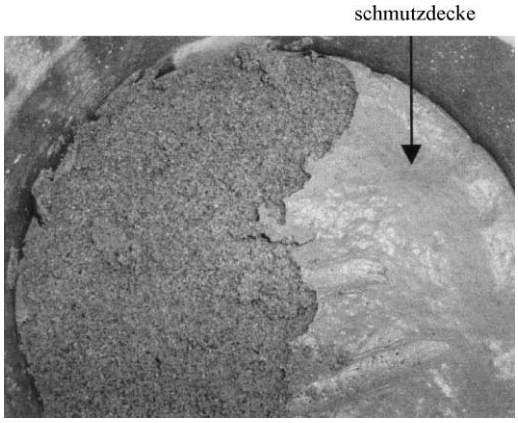

Figure 2 | Scraping of the *schmutzdecke*.

sterile distilled water and in a sterile 10% sodium thiosulfate solution. The contents were placed in equally disinfected 15-l bowls (Figure 4).

Each layer was washed with 11l of sterile distilled water, as follows: (i) 1 to 2l of sterile distilled water was added; (ii) stirring with the ladle used to remove the layer; (iii) pouring out and storage of the water. The procedure was repeated approximately six times. The water obtained was then homogenized and forwarded for analysis. This extraction procedure is supported by published studies (Cleven 2004; Wang *et al.* 2004; Baek *et al.* 2005).

After the entire filter media was removed, the supporting layer was washed by high-pressure upflow dechlorinated water injection. This washing was only interrupted when the effluent from the wash achieved a very low turbidity.

After completion of all procedures, the washed sand was replaced in the filter and the run was resumed, under the same conditions, and a second sample was taken, which was performed in the same manner.

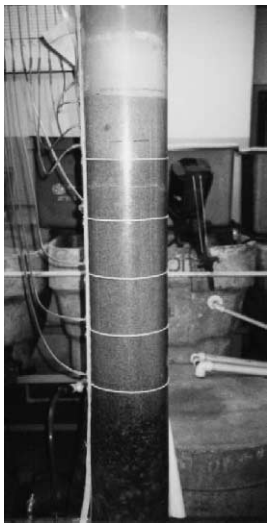

Figure 3 | Filter divided into five 0.15 m layers.

Figure 4 | Washing of the sand layers.

Monitoring

Turbidity, apparent and true colour were evaluated daily both in the raw water and in the effluent from the filters. Turbidity, apparent and true colour, pH and solids were evaluated in the sand layers. Total chlorine and free chlorine were also evaluated in the raw water, for verification of the efficiency of the activated carbon filters in removing this component.

The following microbiological parameters were evaluated in the raw water, in the effluent, in the sand layers, and in the *schmutzdecke* of the DSSF:

- total coliforms
- *Escherichia coli*
- *Clostridium perfringens* and anaerobic bacteria spores
- *Bacillus subtilis* and aerobic bacteria spores
- total heterotrophic bacteria (HPC)
- *Cryptosporidium* sp. oocysts and *Giardia* sp cysts

The turbidity, colour, pH and solids analyses were performed according to *Standard Methods* (1998). Total coliform and *E. coli* were analysed by the enzyme substrate test, by using the Colilert™ culture media. The

total heterotrophic bacteria were analysed by the pour-plate technique, described in section 9215B of *Standard Methods* (1998). *C. perfringens* and anaerobic bacteria spores and *B. subtilis* and aerobic bacteria spores were analysed by the filtering membrane technique, using respectively the modified agar mCP media (USEPA 1996) and a no selective nutrient agar containing 0.01 μg l^{-1} of trypan blue dye and 0.1% soluble starch (Nieminski *et al.* 2000).

For analysis of protozoa, the water samples were concentrated according to the calcium carbonate flocculation technique described by Vesey *et al.* (1993). The direct immunofluorescence technique, by using the Merifluor® C/G kit (Cod. 250050), was applied for identification of *Cryptosporidium* and *Giardia* in the concentrated samples.

For analysis of the bacteriological parameters in the *schmutzdecke*, 2 g of the material were collected and resuspended in 250 ml of sterile distilled water in each experiment. After the homogenization of this suspension, the analyses were performed by the same techniques as those described above for the water samples. The protozoa were analysed by an adaptation of the technique described by Timms *et al.* (1995). Ten grams of the *schmutzdecke* were weighed in a centrifuge tube and 40 ml of Tween 80 solution at a concentration of 0.01% was added. The mixture was agitated in a vortex for 5 minutes. After this period, the sample remained at rest for some minutes for all the sand to sediment out at the bottom of the tube, and then all the supernatant was removed and centrifuged for 15 minutes at 2,200 × g. After centrifugation, the supernatant was removed, maintaining a final volume of 10 ml, which was submitted to the immunofluorescence analysis described for water samples.

Data analysis

The data obtained for the different situations assessed, two for each layer, were evaluated qualitatively and by graphic comparison, in view of the small amount of data available. The association between the physicochemical and microbiological indicators and the protozoa was made by means of the Spearman correlation.

RESULTS AND DISCUSSION

Experimental water

The characteristics of the experimental water are presented in Table 2. Previous research with this water showed an average water temperature of 26°C (20–32°C).

The removal of protozoa

Due to the low concentration of *Giardia* in the influent, just two positive results were obtained for these protozoa inside the filter media.

Figure 5 shows the averages of the distribution of *Cryptosporidium* oocysts inside the filter media of the DSSF between the two duplicate experiments.

For both filtration rates, the first layer always contained a higher number of oocysts, independently of the flow or rate assessed. It is also possible to observe a decreasing number from the first to the last layer, especially at the lower filtration rate, and a systematic absence of oocysts in the last layer (0.60–0.75 m).

For layers 2, 3 and 4 and the two rates assessed, the amount of *Cryptosporidium* was lower for the second day of the run (4 and 8 days), which might indicate some mechanism of 'destruction' of these oocysts inside the filter media, such as predation, for example. These oocysts were unable to develop in the environment, and behaved as inert particles inside the filter media, remaining attached to the biofilm, where they were exposed to the most adverse environmental conditions. These actions together (production of inhibiting substances, for example) may contribute to the oocysts being retained inside the sand layer. They may also lose their viability and infective capacity, emphasizing the disinfective performance of these filters. Although the viability of the oocysts inside the sand media and in the effluent has not been the object of this study, it is worth mentioning that changes to the viability imply modifications in the oocysts' surface characteristics, which would interfere with their interaction with the sand media (Drozd & Schwartzbrod 1996).

The penetration of oocysts is larger at the 6 $m^3 m^{-2} \cdot d$ rate than at the 3 $m^3 m^{-2} \cdot d$ rate, evidenced by the higher amount on the lower layers, especially in the initial analysis (second day of the run). Higher filtration rates imply higher

Table 2 | Characteristics of the experimental water

Parameter	Unit	Average	Median	Minimum	Maximum	Inferior quartile	Superior quartile	Standard deviation
Microorganism								
TC	MPN $100\,ml^{-1}$	5.41×10^3	1.45×10^3	9.00	4.10×10^4	4.10×10	$3{,}90 \times 10^3$	1.07×10^4
EC	MPN $100\,ml^{-1}$	9.33×10^2	1.65×10^2	2.00	5.60×10^3	9	$6{,}60 \times 10^2$	1.61×10^3
ANBS	CFU $100\,ml^{-1}$	1.45×10	9.00	0.00	7.90×10	3	2.20×10	1.95×10
CP	CFU $100\,ml^{-1}$	9.35	3.00	0.00	7.90×10	0	$1{,}00 \times 10$	1.88×10
ABS	CFU $100\,ml^{-1}$	1.49×10^5	5.50×10^3	1.00×10	1.10×10^6	1.00×10^3	2.10×10^5	2.80×10^5
BS	CFU $100\,ml^{-1}$	1.88×10^4	1.00×10^3	1.00×10	1.40×10^5	1.00×10^3	$1{,}0 \times 10^4$	4.15×10^4
HPC	CFU $100\,ml^{-1}$	7.07×10^7	1.70×10^7	1.00×10^5	4.10×10^8	1.60×10^6	7.30×10^7	1.18×10^8
C	oocysts l^{-1}	2.01×10^2	1.42×10^2	2.90×10	5.40×10^2	$7{,}00 \times 10$	$3{,}22 \times 10^2$	1.61×10^2
G	cysts l^{-1}	2.82	0.00	0.00	2.50×10	0	0	7.98
Physicochemical								
pH		6.76	6.67	6.20	7.56	6.41	7.00	0.39
T	NTU	15.58	14.15	8.02	38.80	12.30	17.90	6.75
AC	CU	100.17	94.50	59.00	206.00	83.00	112.00	34.00
TCL	CU	12.61	10.50	6.00	23.00	9.00	17.00	5.29
TS	mg l^{-1}	3.38×10^3	4.34×10^3	1.43×10^2	1.13×10^4	$2{,}29 \times 10^2$	$5{,}40 \times 10^3$	3.27×10^3
TVS	mg l^{-1}	3.26×10^3	4.38×10^3	2.10×10	1.10×10^4	8.90×10	$5{,}34 \times 10^3$	3.26×10^3
TFS	mg l^{-1}	1.35×10^2	1.26×10^2	9.00	3.37×10^2	$5{,}60 \times 10$	2.07×10^2	8.84×10
TSS	mg l^{-1}	3.97×10	3.00×10	1.80×10	1.17×10^2	2.60×10	4.60×10	2.52×10
VSS	mg l^{-1}	9.17	7.00	2.00	2.90×10	5	1.10×10	7.34
FSS	mg l^{-1}	4.03×10	2.40×10	1.00×10	2.14×10^2	1.60×10	4.00×10	4.83×10
TDS	mg l^{-1}	3.11×10^3	2.31×10^3	8.10×10	1.12×10^4	1.46×10^2	5.38×10^3	3.34×10^3
VDS	mg l^{-1}	3.26×10^3	4.37×10^3	1.60×10	1.10×10^4	1.27×10^2	5.34×10^3	3.26×10^3
FDS	mg l^{-1}	1.21×10^2	1.07×10^2	9.00	3.24×10^2	$5{,}70 \times 10$	1.69×10^2	8.60×10

TC, total coliforms; T, turbidity; EC, *Escherichia coli*, AC, apparent colour; ANBS, anaerobic bacteria spores; TCL, true colour; CP, *Clostridium perfringens*; TS, total solids; ABS, aerobic bacteria spores; TVS, total volatile solids; BS, *Bacillus subtilis*; TFS, total fixed solids; HPC, heterotrophic plate counts; TSS, total suspended solids; C, *Cryptosporidium*; VSS, volatile suspended solids; G, *Giardia*; FSS, fixed suspended solids; TDS, total dissolved solids; VDS, volatile dissolved solids; FDS, fixed dissolved solids.

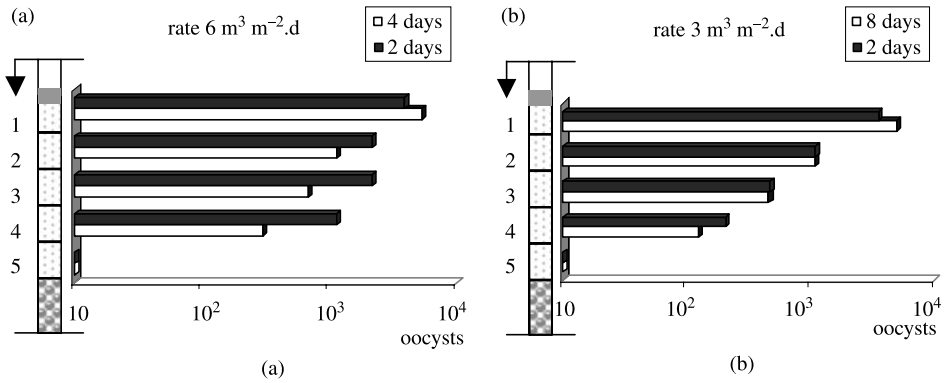

Figure 5 | Amount of *Cryptosporidium* in the DSSF layers at the two rates (a) $6\,m^3\,m^{-2}\cdot$ or $\cdot d$ and (b) $3\,m^3\,m^{-2}\cdot$ or $\cdot d$ at the two times of each run (depth of each layer: 0.15 m).

velocities inside the intergranular spaces, resulting in more difficult adhesion of the particles and, consequently, of the oocysts, especially when the biofilm on the sand layer is not well formed. The fact that the oocysts load is double at the $6\,m^3\,m^{-2}\cdot d$ rate helps to explain this mechanism. Several authors have related the influent concentration of micro-organisms to the effluent concentration in slow sand filters, when these had not reached the ideal biological maturation, due to low resistance to the penetration of organisms because of the absence of a stabilized biofilm (Fox *et al.* 1984; Bellamy *et al.* 1985a).

Figure 6 represents the distribution of *Cryptosporidium* oocysts on the USSF layers. A higher accumulation of oocysts is also observed in layer 1 in comparison with the other layers, with layer 5 always free from oocysts. The large difference is that no *Cryptosporidium* oocyst was found on layer 4 when the filtration rate of $3\,m^3\,m^{-2}\cdot d$ was applied. As mentioned before, lower filtration rates imply lower speeds in the interstices of the filter media, which facilitates the action of physical and chemical retention mechanisms, as well as the biological action of the filter media. However, this argument could be applied to the two flows assessed, indicating that some additional factor could be responsible for this lower penetration of oocysts in the filter media of the USSF at a lower rate. It might be an indication that the support layer plays an active role in the removal of suspended solids during the filtration process, thus reducing the solid load to the filter media, and consequently reducing the penetration.

Figure 6 shows slightly higher concentrations in the layers at the time of the first analysis, in the runs with a lower rate of filtration. Two hypotheses may be considered for this fact: (a) as the penetration inside the filter media was lower, the oocysts that entered the filter were distributed over the three initial layers, in a more uniform manner than at the rate of $6\,m^3\,m^{-2}\cdot d$; (b) the concentration of oocysts in the raw water was higher in the two runs in which the rate of $3\,m^3\,m^{-2}\cdot d$ was evaluated (lowest rate: 262 oocysts l^{-1}; highest rate: 121 oocysts l^{-1}).

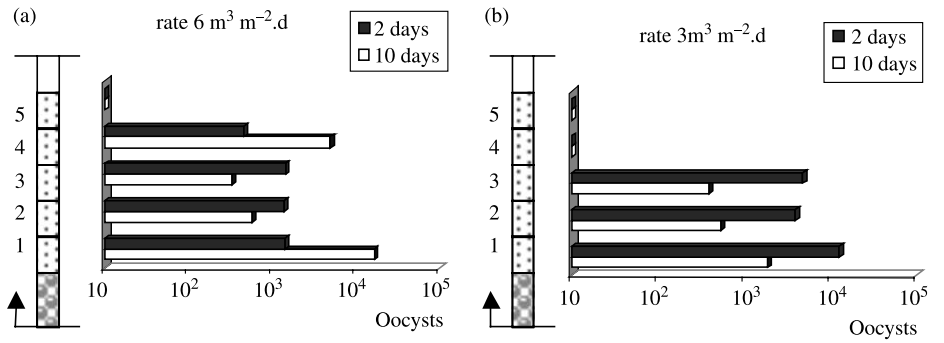

Figure 6 | Amount of *Cryptosporidium* in the USSF layers at the two rates (a) $6\,m^3\,m^{-2}\cdot$ or $\cdot d$ and (b) $3\,m^3\,m^{-2}\cdot$ or $\cdot d$) at the two times of each run (depth of each layer: 0.15 m).

Table 3 shows the relative distribution of oocysts inside the filter media of both filters, in all runs performed.

In the downflow filtration, the removal of oocysts in the initial 0.45 m has always been higher than 80%. In the upflow filter, the lowest removal rate was of approximately 75%, corresponding to the runs in which the filtration rate was higher. The additional removal occurring on layer 4 (0.45 – 0.60 m) should be emphasized, in the cases in which the initial 0.45 m did not remove the total of oocysts influent to the filter. By evaluating the removal of particles ranging between 7 and 12 μm inside a downflow slow sand filter, Fox *et al.* (1984) observed that the most significant removal occurred in the initial 0.07 m. This analysis was performed in the interstitial water, collected at the points used for measurement of head loss, at a flow rate of 2 ml min^{-1}, in order not to disturb the filtration process. The particle size chosen by the authors was based on the dimension of the *Giardia* cysts, larger than the *Cryptosporidium* oocysts, which would allow the assumption of a larger penetration of oocysts compared with that of cysts. In addition, it is known that, for slow sand filters, the particle counts should be carefully interpreted, since a varied microfauna can be found in the media, comprising organisms of different dimensions, from oligochaetes to bacteria, and even viruses, too small to be detected by particle counters (Lloyd 1973).

Timms *et al.* (1995), by evaluating *Cryptosporidium* oocysts inside slow sand filters, found a maximum penetration depth of 0.025 m. However, several differences can be observed between their work and this. According to the authors, the analysed plant, in demonstration scale, was fed by the same influent as that of the real plant, previously treated by three horizontal flow prefilters. Prior to adding oocysts to the raw water, the filter operated normally for five weeks, and the analysis was only performed after that, when there was no more *E. coli* in the effluent. The experiment for verification of the in-depth removal lasted 6 hours: the effluent and the raw water were evaluated every 15 minutes and, at the end of the experiment, small aliquots of sand were analysed for the presence of oocysts of the protozoan at several depths.

Table 4 shows the *Cryptosporidium* mass balance for all assessed situations, calculated in two forms: (i) by considering the concentration of raw water on the day of the analysis; (ii) by considering the average concentration of raw water in all experiments.

In analysing the table, it is noticed that it was not possible to complete the mass balance, in which calculations seem to overestimate the effluent of the runs when obtained by subtraction, in comparison with the effluent obtained from the analyses performed for each run. This difference in the calculations can have several explanations, as follows:

(i) The supposed biological control inside the filter media, responsible for the reduced number of oocysts present on the analysed layers
(ii) Analytical difficulties in detecting protozoa cysts and oocysts, which might contribute to their underestimation in the media
(iii) The raw water used in the 4-, 8- and 10-day runs may have presented some variation, even though it was always contaminated with the same amount of oocyst stock suspension, due to variations in the wastewater concentration

Cryptosporidium oocysts have only been detected in the effluent in two situations:

- Upflow filter, filtration rate of 6 $m^3 m^{-2}$ ·d, two-day analysis
- Downflow filter, filtration rate of 6 $m^3 m^{-2}$ ·d, four-day analysis

In both situations, the filtration rate was the highest, and more unfavourable to the generation of an improved effluent quality. Regarding the upflow filter, it is known that the initial operation period is critical, and in that period the quality of the effluent is much worse than that of the DSSF. However, the presence of protozoa in the effluent of the downflow filter operating for four days indicates that the filter was not sufficiently mature for a consistent removal of pathogens, even though producing a stable effluent in terms of turbidity.

Regarding the *schmutzdecke*, Table 3 shows that it only played a relevant role in removing oocysts with more advanced development (R3-2D run). For the other evaluated runs, the maximum percentage the *schmutzdecke* was able to remove was 5.96%. As oocysts were detected in the effluent only once, it is clear that the filter media as a whole acts directly on the removal of protozoa.

Table 3 | Distribution of *Cryptosporidium* on the layers of the filter media (DSSF and USSF) and in the *schmutzdecke* (DSSF) (absolute values and percentages)

	Absolute values per layer							Percentage *						
Run	**Sh**	**1**	**2**	**3**	**4**	**5**	**Total (oocysts)**	**Sh**	**1**	**2**	**3**	**45 cm**	**4**	**5**
R1-1D	5.78×10^{1}	4.95×10^{3}	1.80×10^{3}	4.36×10^{3}	2.31×10^{3}	ND	1.35×10^{4}	0.43	36.73	13.36	32.35	82.86	17.14	0.00
R1-2D	1.29×10^{2}	8.80×10^{3}	2.31×10^{2}	8.25×10^{2}	6.05×10^{2}	ND	1.06×10^{4}	1.22	83.10	2.18	7.79	94.29	5.71	0.00
R2-1D	7.80	2.20×10^{3}	7.26×10^{3}	5.10×10^{3}	ND	ND	1.46×10^{4}	0.05	15.10	49.84	35.01	100.00	0.00	0.00
R2-2D	1.31×10^{2}	1.90×10^{3}	1.60×10^{3}	4.15×10^{2}	2.48×10^{2}	ND	4.29×10^{3}	3.04	44.25	37.27	9.67	94.22	5.78	0.00
R3-1D	3.24×10^{1}	2.86×10^{3}	ND	ND	ND	ND	2.89×10^{3}	1.12	98.88	0.00	0.00	100.00	0.00	0.00
R3-2D	4.25×10^{3}	2.00×10^{3}	ND	5.50×10^{2}	ND	ND	7.07×10^{3}	63.94	28.28	0.00	7.78	100.00	0.00	0.00
R4-1D	4.66×10^{2}	5.01×10^{3}	1.49×10^{3}	4.40×10^{2}	4.13×10^{2}	ND	7.82×10^{3}	5.96	64.07	19.06	5.63	94.72	5.28	0.00
R4-2D	6.07×10^{1}	2.00×10^{3}	5.50×10^{2}	2.09×10^{2}	ND	ND	2.82×10^{3}	2.15	70.93	19.51	7.41	100.00	0.00	0.00
R1-1U		1.90×10^{3}	2.34×10^{3}	2.15×10^{3}	ND	ND	6.39×10^{3}		29.73	36.62	33.65	100.00	0.00	0.00
R1-1Ub		1.16×10^{3}	1.32×10^{3}	1.21×10^{3}	6.05×10^{2}	ND	4.30×10^{3}		27.01	30.73	28.17	85.91	14.09	0.00
R1-2U		2.97×10^{4}	ND	ND	9.90×10^{3}	ND	3.96×10^{4}		75.00	0.00	0.00	75.00	25.00	0.00
R2-1U		2.40×10^{4}	7.15×10^{3}	8.91×10^{3}	ND	ND	4.01×10^{4}		59.91	17.85	22.24	100.00	0.00	0.00
R2-2U		2.04×10^{3}	9.35×10^{2}	7.04×10^{2}	ND	ND	3.68×10^{3}		55.45	25.41	19.14	100.00	0.00	0.00
R3-1U		1.21×10^{3}	4.95×10^{2}	9.90×10^{2}	7.70×10^{2}	ND	3.47×10^{3}		34.92	14.29	28.57	77.78	22.22	0.00
R3-2U		3.30×10^{3}	1.16×10^{3}	6.60×10^{2}	ND	ND	5.12×10^{3}		64.45	22.66	12.89	100.00	0.00	0.00
R4-1U		1.36×10^{3}	7.15×10^{2}	6.60×10^{2}	ND	ND	2.74×10^{3}		49.73	26.14	24.13	100.00	0.00	0.00
R4-2U		1.34×10^{3}	1.54×10^{3}	9.02×10^{2}	ND	ND	3.78×10^{3}		35.43	40.72	23.85	100.00	0.00	0.00

Sh–*schmutzdecke*.
ND–not detected.
R1-1Ub–b; repeated run.
*Percentage retained in each layer relative to the total retained inside the filter media, emphasizing the total accumulated in the initial 0.45 m of the media, corresponding to layers 1, 2 and 3, including the *schmutzdecke* in the DSSF.

Table 4 | *Cryptosporidium* mass balance in the DSSF and in the USSF, for all runs

	Raw water			Total effluent [4]		Effluent (no. l^{-1})[5]		
Run	**Punctual analysis [1]**	**Average [2]**	**Number inside filter [3]**	**Punctual**	**Average**	**Punctual**	**Average**	**Effluent (actual aqueous sample) (no. l^{-1})**
R1-1D	1.21×10^5	7.53×10^4	1.34×10^4	1.07×10^5	6.19×10^4	2.86×10^2	1.65×10^2	0
R1-2D	1.35×10^5	1.51×10^5	1.27×10^4	1.22×10^5	1.38×10^5	1.63×10^2	1.84×10^2	4.50×10^{-1}
R2-1D	7.21×10^4	3.76×10^4	8.04×10^1	7.20×10^4	3.75×10^4	3.85×10^2	2.01×10^2	0
R2-2D	5.25×10^4	1.51×10^5	4.65×10^3	4.78×10^4	1.46×10^5	6.38×10^1	1.95×10^2	0
R3-1D	1.09×10^4	7.53×10^4	2.89×10^3	7.97×10^3	7.24×10^4	2.13×10^1	1.93×10^2	0
R3-2D	2.62×10^4	1.51×10^5	5.07×10^3	2.11×10^4	1.45×10^5	2.82×10^1	1.94×10^2	0
R4-1D	1.20×10^4	3.76×10^4	7.81×10^3	4.17×10^3	2.98×10^4	2.23×10^1	1.59×10^2	0
R4-2D		1.51×10^5	8.20×10^2	5.20×10^2	1.50×10^5	-1.10×10^0	2.00×10^2	0
R1-1A	4.23×10^4	7.53×10^4	6.38×10^4	-2.15×10^4	1.15×10^4	-5.74×10^1	3.06×10^1	0
R1-1Ab	1.21×10^5	$7.53x10^4$	5.59×10^4	6.47×10^4	1.94×10^4	1.73×10^2	5.17×10^1	4.00×10^0
R1-2A	6.03×10^5	3.76×10^5	3.96×10^4	5.63×10^5	3.37×10^5	3.01×10^2	1.80×10^2	0
R2-1A	6.03×10^4	3.76×10^4	4.00×10^4	2.03×10^4	-2.37×10^3	1.08×10^2	-1.27×10^1	0
R2-2A	3.01×10^5	1.88×10^5	3.67×10^3	2.98×10^5	1.84×10^5	3.18×10^2	1.97×10^2	0
R3-1A	1.21×10^5	7.53×10^4	3.47×10^3	1.17×10^5	7.18×10^4	3.13×10^2	1.92×10^2	0
R3-2A	6.03×10^5	3.76×10^5	5.12×10^3	5.98×10^5	3.71×10^5	3.19×10^2	1.98×10^2	0
R4-1A	6.03×10^4	3.76×10^4	2.74×10^3	5.75×10^4	3.49×10^4	3.07×10^2	1.86×10^2	0
R4-2A	3.01×10^5	1.88×10^5	3.78×10^3	2.98×10^5	1.84×10^5	3.18×10^2	1.97×10^2	0

[1]Total volume that passed through the filter (flow rate × time) × concentration of the raw water in the analysis of the run.
[2]Total volume that passed through the filter (flow rate × time) × average concentration of the raw water.
[3]Total amount on all layers (1 to 5). The *schmutzdecke* was also added to the DSSF.
[4]Subtraction: Raw water – contained in the filte.r
[5]Estimated effluent concentration (total effluent/total volume).

Association between indicators and protozoa

Table 5 shows the Spearman correlation values for the associations. As observed, the only statistically significant association was between *Cryptosporidium* and *Giardia*. As practically no *Giardia* cysts were detected in the raw water and inside the filters, it is difficult to make evaluations about this relationship inside the filter media. Vieira *et al.* (2002), by evaluating the presence of these two protozoa in sewage, noticed a similarity in their occurrence profile. Nieminski *et al.* (2000) found no significant correlation between the occurrence of *Cryptosporidium* and *Giardia*, both in raw water and in effluents from rapid filtration plants. However, Bastos *et al.* (2004) verified that the presence of the two protozoa in surface water sources in Brazil is statistically associated.

By applying the ranges adopted in statistics texts for classification of the R values, it is noticed that none of the indicators used in the attempt to represent the behaviour of the *Cryptosporidium* oocysts inside the filter media showed a relationship better than 'very weak' with this protozoan. This level of correlation was observed both for the traditional treatment efficiency indicators and for the new microbiological indicators proposed.

Aiming at finding a disinfection efficiency indicator more competent than the total coliforms, Barbeau *et al.* (1999) observed that the aerobic bacteria spores presented the same behaviour as the *Giardia* cysts in disinfection. In spite of having reported the aerobic bacteria spores as good indicators of the efficiency and quality of the treatment, Nieminski *et al.* (2000) did not get satisfactory R^2 values in the simple regression performed between all these indicators and the *Cryptosporidium* oocysts.

The absence of a relationship between the assessed protozoa and the turbidity should be emphasized, since the turbidity has been indicated as a sentinel parameter for the removal of protozoa. In water treatment involving rapid filtration, the maximum effluent turbidity recommended by the Brazilian legislation is 0.5 NTU, which could ensure potable water suitable for human consumption with respect to the presence of protozoa. The absence of a correlation between this parameter and the *Cryptosporidium* oocysts suggests the need for supplementary studies about this relationship and on how it really influences the effluent from slow sand filters.

Table 5 | Spearman correlation among the *Cryptosporidium* oocysts

Indicator	Valid N	Spearman R	t (N-2)	p-level	Indicator	Valid N	Spearman R	t (N-2)	p-level
TC	78	0.13	1.18	0.24	TFS	78	−0.03	−0.23	0.82
EC	78	0.09	0.77	0.44	TSS	78	0.12	1.03	0.31
ANBS	73	0.11	0.93	0.36	VSS	78	0.03	0.30	0.77
CP	73	0.14	1.23	0.22	FSS	78	0.13	1.11	0.27
ABS	78	0.12	1.04	0.30	TDS	78	0.08	0.67	0.50
BS	73	0.07	0.56	0.58	VDS	78	0.08	0.69	0.49
HPC	68	0.04	0.38	0.71	FDS	78	−0.04	−0.35	0.73
G	78	0.35	3.30	0.001	PH	78	0.16	1.39	0.17
TS	78	0.05	0.40	0.69	T	78	0.14	1.21	0.23
TVS	78	0.06	0.52	0.60	TCL	78	0.07	0.61	0.54

TC, total coliforms; T, turbidity; EC, *Escherichia coli*, ANBS, anaerobic bacteria spores; TCL, true colour; CP, *Clostridium perfringens*; TS, total solids; ABS, aerobic bacteria spores; TVS, total volatile solids; BS, *Bacillus subtilis*; TFS, total fixed solids; HPC, heterotrophic plate counts; TSS, total suspended solids; VSS, volatile suspended solids; G, *Giardia*; FSS, fixed suspended solids; TDS, total dissolved solids; VDS, volatile dissolved solids; FDS, fixed dissolved solids.

CONCLUSIONS

The work carried out allows us to draw the following conclusions:

- *Cryptosporidium* oocysts present a similar behaviour for the higher filtration rate ($6\,m^3\,m^{-2}\cdot d$) in both flows assessed. For lower filtration rates ($3\,m^3\,m^{-2}\cdot d$), the penetration of these oocysts is higher in the downflow slow sand filter, reaching 0.60 m deep. In the upflow slow filter it is limited to the initial 0.45 m of the filter media.
- The downflow filter presented no difference between the retention of the oocysts in both rates assessed. For the upflow, it should be noticed that the penetration of these organisms is lower in the lower filtration rate than in the higher filtration rate.
- The results show that there is no accumulation of oocysts inside the filter media as the run develops, especially in the deeper layers (0.15–0.60 m), suggesting the existence of some type of control of these microorganisms inside the filters.
- The *schmutzdecke* seems to play an effective role in the removal of protozoa only when it is well developed, affecting the retention in a differentiated manner in the assessed situations, but without a well-defined standard for this influence.
- Weak relationships were found between the traditional water quality and treatment efficiency indicators – turbidity, total coliforms and *E. coli* – and the *Cryptosporidium* oocysts inside the filter media. In the same manner, the new microbiological indicators proposed did not present a relationship with the oocysts either, suggesting once again the need to research indicators that really represent the retention of these pathogens during treatment.

The recommendations for works to be developed in the future are as follows:

- The evaluation of the viability of the oocysts in the filter media and in the *schmutzdecke*, in order to estimate the real disinfection capacity of this type of filtration regarding these pathogens.
- The validation of the relationship between protozoa cysts and oocysts and the turbidity parameter for both slow sand filtration and rapid filtration, either by conventional treatment or by direct filtration.
- Supplementary study of the free living protozoa and algae inside the filter media of the slow sand filters, simultaneously with the study of the dynamics of pathogens and indicators, aiming at aiding the discussion on their behaviour under different operational conditions.

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