

Nitrification, denitrification and ammonification in point-of-use biosand filters in rural Cambodia

Heather M. Murphy, Edward A. McBean and Khosrow Farahbakhsh

ABSTRACT

In order to address the United Nations Millennium Development Goal (MDG) target #7 for water and sanitation, the World Health Organization (WHO) has identified point-of-use (POU) water treatment technologies as an option for providing safe water to households. The BioSand filter (BSF) is a commonly used POU system that has been implemented in Cambodia and over 20 countries worldwide. While the health benefits of using a BSF in terms of reduction of diarrheal disease have been fairly well documented, little research has focused on the ability of this technology to treat for other contaminants that could pose health concerns. To address these concerns, a study was developed to evaluate this technology in rural Cambodia in terms of microbiological and chemical quality of the treated water. The study revealed that simultaneous nitrification and denitrification is occurring inside the BioSand filters. Nitrite concentrations in treated water consistently exceeded WHO guidelines. Seventeen of 20 filters on average did not meet the $3.0\text{mg l}^{-1}\text{NO}_2^-$ guideline and the combined nitrate-nitrite guideline ratio of 1. Denitrification seemed to predominate when BSFs were fed surface water. In addition, nitrate-ammonification occurred in some filters fed surface water, causing increases in ammonia in treated water.

Key words | biosand filters, Cambodia, denitrification, household water treatment, nitrification, point-of-use water treatment

Heather M. Murphy (corresponding author)
Edward A. McBean
Khosrow Farahbakhsh
School of Engineering,
University of Guelph,
50 Stone Rd. East,
Guelph,
Canada N1G 2W1
Tel.: +1 (519) 824-4120 ext. 52231
Fax: +1 (519) 836-0227
E-mail: murphy@uoguelph.ca

INTRODUCTION

Today, 1.1 billion people do not have access to a safe drinking water source (WHO/UNICEF 2005; UN 2006). According to Smout (2000, p. 27) 'water related diseases are the single largest cause of human sickness and death', as there are 3.3 million deaths annually as a result of diarrheal disease. In order to address the United Nations Millennium Development Goal (MDG) target #7 for water and sanitation, the World Health Organization (WHO) has identified point-of-use (POU) water treatment technologies as an option for providing safe water to households (Sobsey 2002; Sobsey *et al.* 2008). The BioSand filter (BSF) is a commonly used POU system that has been implemented in Cambodia and over 20 countries worldwide (Duke *et al.* 2006).

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While the health benefits of using a BioSand filter in terms of reduction of diarrheal disease have been fairly well documented, little research has focused on the ability of this technology to treat for other contaminants that pose health concerns, including the potential for formation of contaminants as a result of POU treatment. To date, there are few refereed publications that critically evaluate the overall performance of these systems in the field (Lantagne *et al.* 2009). The bulk of the research conducted up to now has been on the health impact in terms of diarrheal disease reduction that may be attributed to the use of these technologies at the household level. Liang *et al.* (in press) have found up to 44% reduction of diarrheal disease in households that use a BioSand filter compared with those that do not.

In order to address these concerns, a study was developed to evaluate POU BioSand filters in terms of microbiological and chemical quality of the treated water. The research was conducted in rural Cambodia on various Cambodian source waters. Given that the BioSand filter was adapted from the design of a traditional slow sand filter, it operates biologically, and thus there is a potential for various microbial processes to occur inside the filter. The impact of this biological activity on water quality has not been closely examined. This paper seeks to address this concern by:

- examining the potential for nitrification and/or denitrification processes to occur inside the BioSand filter;
- evaluating the effect of various source waters on the filter performance;
- assessing whether or not this filter can meet WHO guidelines for nitrate and nitrite in the treated water and,
- examining the impact of household use and practices on the concentration of nitrate and nitrite in treated water from the BSF.

METHODS

BioSand filter design

The BSF is a household-operated slow sand filter (Figure 1). In the early 1990s, Dr Manz at the University of Calgary adapted the design of a traditional slow sand filter so that it could be operated intermittently and called it the BSF (Buzunis 1995; Palmateer *et al.* 1999). The design of the BSF in Cambodia consists of a concrete frame and locally available crushed rock as the filter media. The rock is crushed to two different sizes: a coarse layer and then a fine layer. The fine layer of crushed rock (sand) makes up the majority of the filter, approximately 46 cm, and has an effective size of between 0.15 and 0.35 mm and a uniformity coefficient of <3 (Samaritan's Purse Canada 2008). To date, approximately 25,000 have been installed throughout Cambodia by two local organizations: Hagar and Cambodia Global Action (CGA).

There are two principal mechanisms that govern the performance of slow sand filters: physical removal mechanisms and biological removal mechanisms. Physical removal occurs when particles present in the water are too

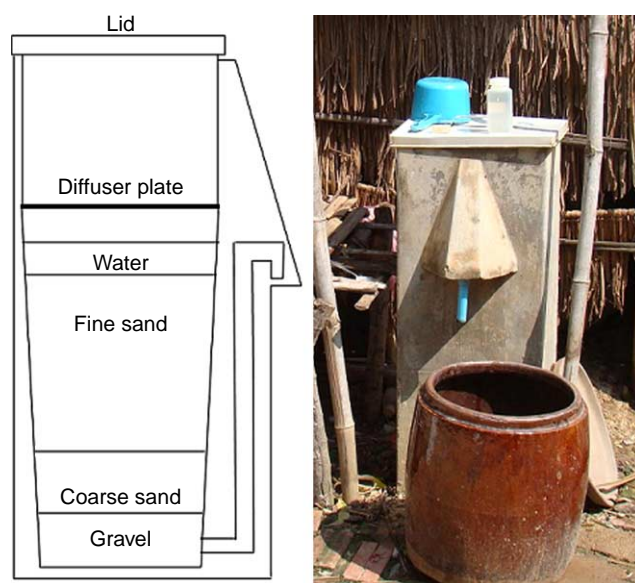


Figure 1 | BioSand filter.

large to pass through the filter bed. Biological removal occurs principally in the top layer of the filter in a biological film known as the 'schmutzdecke'. The schmutzdecke acts as both a fine filter to remove small colloidal particles as well as a biological zone that degrades soluble organics and destroys harmful pathogens (Weber-Shirk & Dick 1997). Although the ability of slow sand filters to remove microorganisms from water supplies is well documented, there is very little literature that demonstrates that BioSand filters have the same capabilities.

There are some key design differences between the two technologies. For instance, slow sand filters are operated continuously with a constant head, while BioSand filters are operated intermittently, with variable head and higher flow rates. The flow rate of BioSand filters ranges from 30 to 40 l/hour. *Escherichia coli* removal has been documented to range between 63 and 99% in a laboratory setting and between 0 and 99.7% in field trials (Duke *et al.* 2006; Earwaker 2006; Stauber *et al.* 2006; Baumgartner *et al.* 2007). The effectiveness of this system on virus removal is not well documented. Elliot *et al.* (2008) observed an average 0.5 log reduction of bacteriophage in lab trials. Bacterial reductions vary from 73.7 to 98.5% in the lab and field depending on operating conditions and filter ripening stage. To the researchers' knowledge, there is no published

work that evaluates the BSF in terms of chemical quality of the treated water including nitrate, nitrite and ammonia.

Household filter selection

A case study approach was used to assess the performance of BSFs in rural Cambodia. Two villages were selected for study in September 2008. The project took place in Kesom and Popeal Kaye villages in Kandal Province, Cambodia (Figure 2). The project was conducted in collaboration with CGA, a local non-governmental organization (NGO) that implements BioSand filters in several provinces in Cambodia. The study design consisted of two parts: 1) initial filter survey; and 2) water quality survey of 20 households over time. The initial study consisted of locating all BioSand filters currently implemented in these two villages. The lead researcher was advised by CGA that a total of 81 filters were installed in both villages. Instead of generating a random sample, an attempt was made to locate all the filters in the communities. The filters ranged in age from 1 to 7 years old. Although 81 filters were located, only 59 were still being used by households at the time of site visit. Once a filter was located, a survey was conducted with the household and water samples were collected from the untreated source

water used for the filter and from the treated water leaving the filter spout. The questionnaire used in the study inquired about filter use, hygiene practices, household demographics and filter maintenance.

From the initial 59 filters, 20 were chosen for part 2 of the study and were examined in more detail over a six-month period. The 20 filters were chosen using a series of criteria. For a household to be included in the study, they needed to be using one of the source waters of interest: surface water or well water. The following criteria were used for excluding households from the study: unwillingness to participate in the study; blending of water sources; using rainwater all year round; having a large number of water jars and therefore potentially storing rainwater for a long period of time; using piped water or bottled water; or using their BSF infrequently. Once the 20 households were selected, they were visited once every two weeks to collect water samples and complete a short questionnaire regarding filter operation and maintenance. These households were visited over a period of six months during the dry season in Cambodia. The dry season was chosen for the study period because during this time households generally use water of poorer quality in their filters such as well and surface water. These water sources contain more contaminants

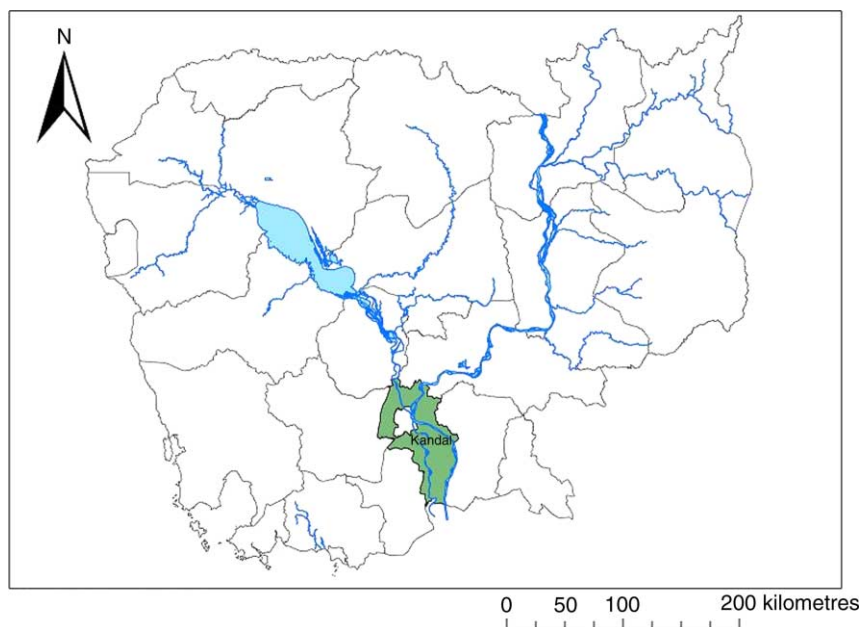


Figure 2 | Kandal Province, Cambodia.

than rainwater, which is considered of higher quality and is frequently used as source water for the filters during the rainy season.

Collection of water samples and analysis

Untreated and treated water samples were collected in sterile autoclaved sample bottles and kept on ice until transported to the Resource Development International Cambodia (RDIC) laboratory where they were analysed within 24 hours for total coliforms (TC), *E. coli*, pH, turbidity, ammonia, nitrate and nitrite. Unfortunately, neither total nitrogen (TN) nor total Kjeldahl nitrogen (TKN) could be measured in Cambodia owing to the unavailability of reagents. Consequently data on the organic nitrogen content in the source water and treated water are not reported in this paper.

Untreated water samples were collected from concrete household water storage containers, surface water sources near the household or directly from wells, depending on how the household collected the raw water to feed their filter. Treated water samples were collected from the spouts of the BioSand filters. In addition, water samples were collected from the treated water storage container used at each household.

Total coliforms and *E. coli* were enumerated using the standard membrane filtration method as outlined in *Standard Methods (2006)*. Samples were filtered aseptically through sterile 0.45- μm filters using a vacuum aspirator. The filters were then transferred using sterile forceps onto pre-dried Oxoid differential coliform agar with BCIG (chromogen 5-bromo-4-chloro-3-indolyl-b-D-glucuronide, used for simultaneous detection of coliforms and *E. coli*) and incubated upside down for 18–24 hours at 37°C. At this time, all pink and blue colonies were enumerated as

coliforms and those colonies that were blue were counted specifically as *E. coli*. All microbiological samples were processed using two serial dilutions and each dilution was processed in duplicate. Ammonia, nitrate and nitrite were measured using a HACH DR/2400 Spectrophotometer, using methods 8155, 8039 and 8153, respectively, as specified in the HACH DR/2400 manual (HACH Company 2004).

Ethics approval

Free and informed consent of the participants was obtained and the study protocol was approved by the Committee for the Protection of Human Subjects: Research Ethics Board at the University of Guelph, Ontario, Canada-Protocol #07OC007, approved 7 March 2008.

RESULTS AND DISCUSSION

The results for this paper will focus on part 2 of the research, the water quality survey of 20 households over time. Results from the preliminary study will be presented separately in another publication. Twenty households, 11 using well water and nine using surface water as their water source for the BioSand filter, were visited for a period of six months. During the six-month period, 11 samples were collected from each household.

General water quality

Average pH, turbidity and *E. coli* data are presented in [Table 1](#) for untreated well and surface water as well as treated water for each source. In the study, nine different well water sources were being used in the community

Table 1 | Average pH, turbidity and *E. coli* concentrations in untreated and treated water for BSFs (values in parentheses represent the range of values observed)

Source water	pH		Turbidity		<i>E. coli</i> (cfu/100 ml)	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Deep well*	7.1 (6.2–8.4)	7.4 (6.7–8.1)	10.4 (0.2–103.5)	0.2 (0.1–1.0)	109 (0–3,585)	13 (0–284)
Surface water†	7.0 (6.2–9.0)	7.6 (6.9–8.6)	9.4 (0.1–52.3)	0.9 (0.1–5.6)	5,842 (0–122,000)	105 (0–1,990)

*Deep well refers to households who use a tube well deeper than 10 m.

†Surface water refers to households using lake or pond water.

(two households shared wells with other households) and nine different surface waters were used. Although some of the surface water samples were taken from the same body of water as other households, all households drew their water from very different locations and all the surface water sources had different water quality characteristics.

The water quality results displayed in Table 1 show that the BSF reduced turbidity and microbiological contamination and increased the pH in the treated water. The pH increase was likely to be a result of calcium carbonate leaching from the concrete frame of the BSF. For the filters that were fed deep well water, reduction in microbiological contamination was significantly lower than filters that were fed surface water. This could be attributed to the fact that the initial well water was relatively free of microorganisms and in these cases the BSF sometimes introduced bacteria into the treated water instead of providing removal. In general, bacterial removal ranged from 0 to 99.9% depending on the influent source water, which is consistent with the findings reported by others (Duke *et al.* 2006; Stauber *et al.* 2009; Liang *et al.* in press).

Although the treated water from the spouts of the BSFs was fairly high in quality in terms of bacterial removal, the water in the storage containers that were used to capture the treated water from the BSF was often highly contaminated. The water collected from these storage containers was almost always of poorer quality than the treated water directly from the spout of the BSF. These containers were often visibly contaminated as shown in Figure 3. The concentrations of *E. coli* in storage containers ranged from 0 to 1,390 CFU/100 ml. When comparing results with WHO guidelines, 4 of 20 treated water storage containers contained water that was on average in the low risk range of 0–10 CFU *E. coli*/100 ml, 11 were in the medium risk range of 11–100 CFU *E. coli*/100 ml and 5 were in the high risk range of 101–1,000 CFU *E. coli*/100 ml.

Comparison of nitrate and nitrite results with WHO guidelines

Some of the most significant findings of this research were the results for nitrite (NO_2^-) and nitrate (NO_3^-). According to the WHO, the guideline values for nitrite and nitrate in drinking water are as follows (WHO 2007):



Figure 3 | BioSand filter storage container.

- i. For short-term (acute) exposure to nitrate for bottle-fed infants, the value should not exceed $50 \text{ mg l}^{-1} \text{ NO}_3^-$
- ii. For short-term exposure to nitrite for bottle-fed infants, the value should not exceed $3.0 \text{ mg l}^{-1} \text{ NO}_2^-$
- iii. For long-term (chronic) exposure to nitrite for all those exposed to the water source, the nitrite value should not exceed $0.2 \text{ mg l}^{-1} \text{ NO}_2^-$ (provisional guideline)
- iv. The combined nitrate-nitrite guideline value should be ≤ 1 :

$$\frac{C_{\text{nitrate}}}{\text{GV}_{\text{nitrate}}} \oplus \frac{C_{\text{nitrite}}}{\text{GV}_{\text{nitrite}}} \leq 1$$

where: C = concentration and GV = guideline value (50 mg l^{-1} for nitrate; 3.0 mg l^{-1} for nitrite).

Source water concentrations for nitrite ranged from 0 to $28.3 \text{ mg l}^{-1} \text{ NO}_2^-$ with an average of 3.32 mg l^{-1} . Nitrate concentrations in source waters were in the range of 0 to $37.7 \text{ mg l}^{-1} \text{ NO}_3^-$ with an average of 2.09 mg l^{-1} . On average, 10 of 20 source waters surpassed a concentration of 3.0 mg l^{-1} for nitrite, and the untreated source waters for all 20 households were in exceedance of the chronic exposure value of 0.2 mg l^{-1} for nitrite. No source water exceeded the guideline value of 50 mg l^{-1} for nitrate. The source waters for this study contained relatively high concentrations of nitrate and nitrite initially because of

several factors. Firstly, the study sites were located in a highly agricultural community where rice farming took place twice during the study period. During this time the fields were flooded and application of fertilizers was common. Concentrations of nitrate and nitrite were highest at the beginning and end of the six-month study period which coincided with the end and beginning of the rainy season as well as the beginning of both rice growing seasons. It is likely that the surface water was impacted by fertilizers as well as human and animal waste given that very few households had access to latrines and few practised animal waste management. Therefore, the combination of all these factors with the increase in rainfall and runoff into surface water bodies is likely to increase the input of nitrogen compounds into the environment. In addition, these seasonal variations in water quality were also observed in the well water sources in the community studied, therefore indicating that the well water in this area was probably under the influence of surface water.

Figures 4 and 5 illustrate the average nitrite and nitrate concentrations, respectively, observed in the treated water from the BSF at each household over the six-month study period. The averages are displayed by the circles on the

graphs, while the bars represent high and low values observed. From Figure 4, one can see that 16 of 20 filters on average exceed or equal the guideline value of $3.0 \text{ mg l}^{-1} \text{ NO}_2^-$. All 20 filters continued to exceed the chronic exposure guideline value of $0.2 \text{ mg l}^{-1} \text{ NO}_2^-$ in the treated water. Although nitrate concentrations increased through the filters from source water to treated water, none produced water with nitrate in excess of 50 mg l^{-1} , therefore complying with WHO recommendations. Table 2 presents the average results for the combined nitrate-nitrite guideline value for each household filter. During the six-month study period, in the untreated source waters, on average, 11 households exceeded the combined nitrate-nitrite guideline value of 1; whereas after treatment an additional 6 filters, and hence a total of 17, did not meet this guideline value.

A probability of exceedance analysis was performed for nitrite. The observed values were ranked from highest to lowest and the probabilities of exceeding the 3.0 mg l^{-1} and 0.2 mg l^{-1} guidelines were determined. The analysis revealed that in treated water produced by the BSFs in this study, there is a 34–38% chance of equalling or exceeding the 3.0 mg l^{-1} nitrite value and a 75% chance of exceeding the 0.2 mg l^{-1} chronic guideline.

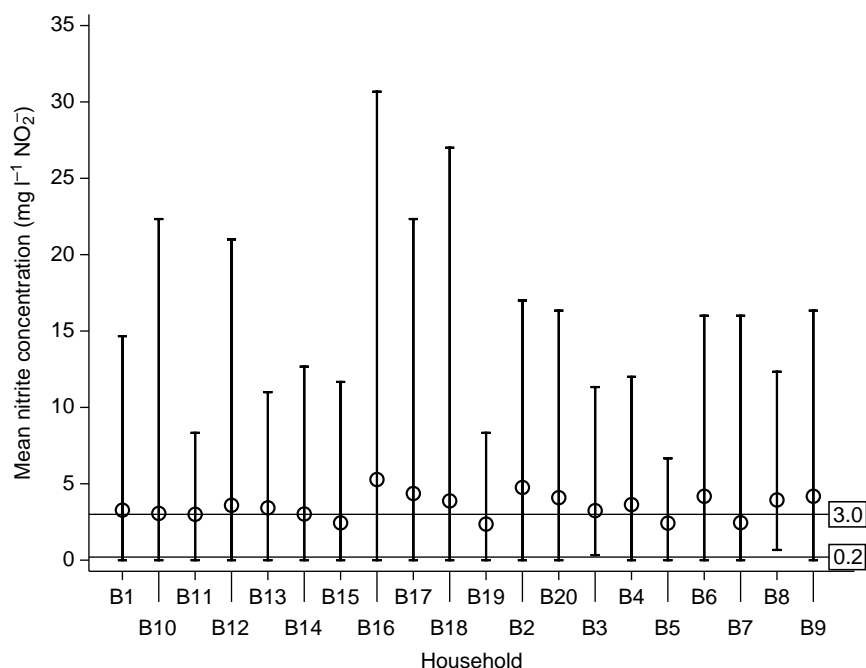


Figure 4 | Mean concentrations of nitrite in treated water for each household filter over the six-month study period (the horizontal lines represent WHO guideline values; error bars represent high and low values observed).

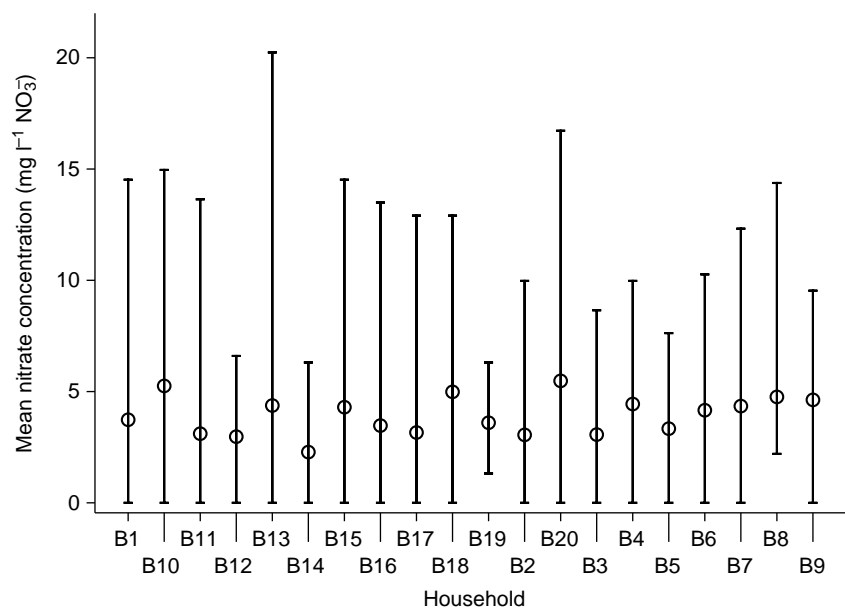


Figure 5 | Mean concentrations of nitrate in treated water for each household filter over the six-month study period (error bars represent high and low values observed).

All of these guideline values are important as nitrite and nitrate may be harmful to infants causing methaemoglobinemia, a condition which occurs when nitrite oxidizes iron in the blood and limits the ability of oxygen to be transported around the body causing veins and skin to appear blue. This condition is often called blue baby syndrome and can affect bottle-fed infants who consume water above the acute exposure guidelines. In addition, if people are exposed to water exceeding the long-term exposure guideline of 0.2 mg l^{-1} nitrite, they may be at risk for heart and lung complications as well as diuresis, a stomach condition that causes increased urination, starchy deposits and haemorrhaging of the spleen (USEPA 2006; WHO 2007). Additionally, some research suggests that brain tumours in children could be caused by prenatal exposure to nitrite as a result of mothers consuming water containing nitrite in excess of guideline values (Forman 2004). However, given the difficulties of conducting epidemiology studies to quantify the health effects of nitrates and nitrites in drinking water, more research is needed before concrete conclusions can be drawn (Forman 2004). To date, still little is known about the long-term effects associated with drinking water containing high concentrations of nitrite and nitrate.

An example of data for one filter: B2

Although Figures 4 and 5 illustrate the final concentrations of nitrate and nitrite in the BSFs studied, they do not give any indication as to what might be occurring inside the filters to cause increases in these compounds. The graphs do not paint a complete picture, as there is considerable variation in nitrate, nitrite and ammonia concentrations from one visit to the next. To examine this issue, Figures 6–8 illustrate an example of the data observed for a single household filter: B2. Deep well water was the source water for filter B2. The household was visited 11 times over the six-month period and Figure 6 presents the influent (source water) and effluent (treated water) concentrations of nitrate, nitrite and ammonia for each site visit. The units are presented in terms of mg l^{-1} of nitrogen for all compounds. Notice that in Figure 6, there are few evident trends; the data varies considerably from site visit to site visit, with the exception of the ammonia data which seems somewhat more stable. In Figure 6, site visits 4 and 10 are highlighted because they are to be examined in more detail in Figures 7 and 8. These sampling events were selected to demonstrate that nitrification and denitrification processes are occurring inside the BSF and to help explain

Table 2 | Average combined nitrate-nitrite guideline values for each household in treated water from the BSFs

Household code	Water source	Average combined nitrate-nitrite values (low-high)*
B1	Deep well	1.17 (0–5.18)
B2	Deep well	1.95 (0–5.78)
B3	Deep well	1.14 (0.15–3.78)
B4	Deep well	1.3 (0.05–4.07)
B5	Deep well	0.87 (0–2.30)
B6	Deep well	1.48 (0–5.39)
B7	Deep well	0.91 (0–5.33)
B8	Deep well	1.53 (0.27–5.51)
B9	Deep well	1.49 (0.01–5.51)
B10	Deep well	1.13 (0.06–7.44)
B11	Surface	1.06 (0.02–2.79)
B12	Surface	1.26 (0–7.05)
B13	Surface	1.23 (0.05–3.75)
B14	Surface	1.05 (0.03–4.34)
B15	Surface	1.18 (0.01–4.03)
B16	Surface	1.63 (0–10.24)
B17	Surface	1.52 (0–7.44)
B18	Surface	1.39 (0.11–9.06)
B19	Deep well	0.86 (0.07–2.80)
B20	Surface	1.47 (0.09–5.78)

*Range of values from lowest value to highest value reported over the six-month sampling period for 11 samples collected at each household.

Note: Numbers in bold indicate households that on average exceeded or equalled guideline values.

the increases and decreases in the relative concentration of various nitrogen compounds.

In Figure 7, note that ammonia decreases from influent to effluent, while nitrite and nitrate increase from influent to effluent. This is an example of nitrification as it follows the following biological processes:

Oxidation of ammonia to nitrite



Oxidation of nitrite to nitrate

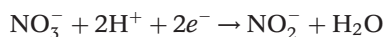


Nitrification is a microbial-driven process by which ammonia-oxidizing bacteria in an oxygen-rich environment convert ammonia to nitrite and then subsequently

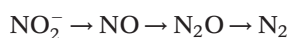
nitrite-oxidizing bacteria convert nitrite to nitrate. The bacteria that perform these processes are *Nitrosomonas* and *Nitrobacter*, respectively (de Vet *et al.* 2009). According to de Vet *et al.* (2009, p. 182), 'nitrification occurs spontaneously in drinking water filters ... under aerobic conditions'. Given that nitrification occurs in an aerobic environment, it is hypothesized that this process takes place at the top of the BSF in the schmutzdecke layer, where oxygen and microbial activity are abundant.

In Figure 8, site visit 10 is highlighted for more detailed study. This figure is an example of denitrification occurring inside the BSF. Notice that as ammonia decreases, nitrite increases and nitrate decreases. In addition, an overall decrease in total nitrogen (TN) was observed during this sampling event. Near the end of the study some samples were sent to Canada for analysis of TN and for this sample TN decreased from 1.36 to 1.16 mg l⁻¹. This indicated that some nitrogen gas may have been produced. The combinations of all these mechanisms follow the microbial processes for denitrification:

Reduction of nitrate to nitrite



Reduction of nitrite to nitrogen gas



Denitrification is a microbial-driven process that takes place in oxygen-deficient environments by which bacteria convert nitrate to nitrite and then further to nitrogen gas. It is hypothesized that in this case, ammonia is decreasing as a result of nitrification occurring at the surface of the BSF. However, as water proceeds through the filter and ultimately 'pauses' for a period of time until the filter is refilled, the water at the bottom of the filter becomes lower in oxygen content, and thus provides ideal conditions for denitrification to occur. It is likely that simultaneous nitrification-denitrification may be occurring inside the BSF as found by Nakhla & Farooq (2003) in slow sand filters in Saudi Arabia. They claim that biological activity occurs not only in the schmutzdecke layer of the filter, but also deep within the filter. These authors reported that denitrification efficiency in the filters studied was much more stable than the nitrification efficiency over time (Nakhla & Farooq 2003). Simultaneous nitrification-denitrification

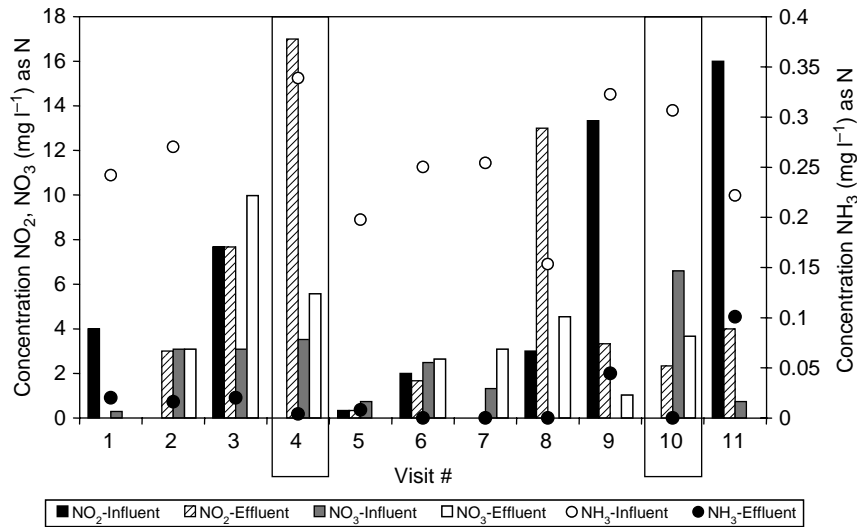


Figure 6 | An example of the nitrate, nitrite and ammonia data collected for filter 'B2'.

is probably occurring during site visit 10 (Figure 8) as first ammonia is oxidized to nitrite and nitrate followed by reduction of nitrate to nitrite and nitrogen gas. This result may help explain the variations in nitrate and nitrite data observed in the current study.

Results from matched-paired *t*-tests conducted for nitrate, nitrite and ammonia

In order to establish if there were any significant trends for changes in nitrate, nitrite and ammonia between untreated

and treated water and to determine whether source water (well or surface water) had any significant effect on these nitrogen compounds, a series of matched paired *t*-tests were performed. First, all nitrate, nitrite and ammonia data were put in terms of change in concentration between untreated and treated water for all data collected (change in concentration = concentration in untreated water – concentration in treated water). These data were then transformed accordingly, using either a log transformation or a Box-Cox transformation so that the data set best

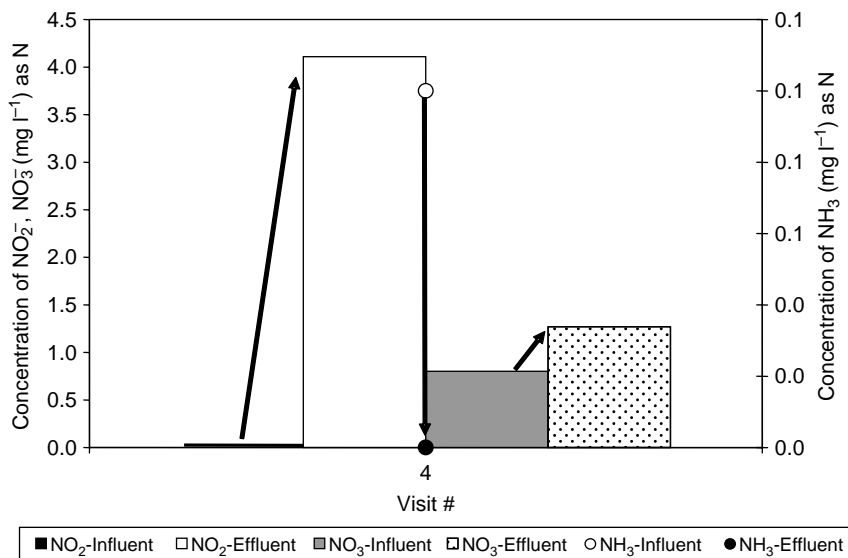


Figure 7 | An example of nitrification on visit #4 in filter 'B2'.

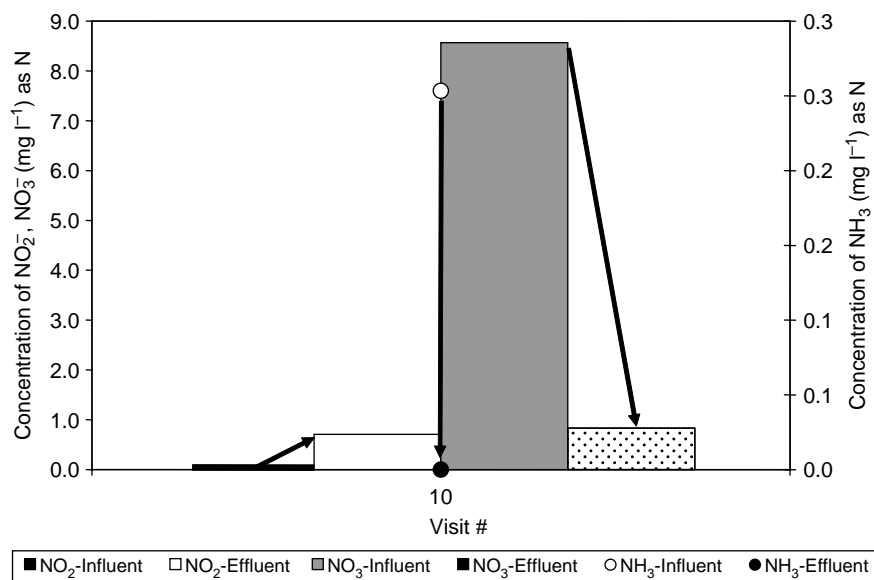


Figure 8 | An example of denitrification on visit #10 in filter 'B2'.

approximated a normal distribution. Then, an analysis of variance (ANOVA) was conducted comparing all surface water households with one another, and then the same was performed for households using well water as their source. The ANOVAs revealed that there were no significant differences between households using the same source water in terms of change in nitrate, nitrite and ammonia concentrations from untreated to treated water (p -values are displayed in Table 3).

Given that there were no significant differences between households using the same source water, households were then grouped into two groups: well water and surface water. The difference in concentrations between untreated and treated water for each parameter were compiled and then transformed using a Box-Cox transformation or log transformation. On the transformed data, a matched paired t -test was performed to see if there were any significant trends for nitrate, nitrite or ammonia from source water to treated BSF effluent. The results of the matched paired t -tests are presented in Table 4. The results reveal that nitrate and nitrite increase on average through the BSF for both water sources. These results were found to be significant at the 99.9% confidence level. Interestingly, the average increase in nitrite is higher for surface water than for well water. Contrarily, the average increase in nitrate was higher for well water than for surface water.

The ammonia concentrations decrease consistently through BSFs fed well water; however they increase slightly on average for filters fed surface water.

These changes in nitrate and nitrite in the BSF further suggest that nitrification-denitrification processes are occurring inside the filters. A higher increase in nitrite and a lower increase in nitrate, for filters fed surface water could indicate that these BSFs are seeing more denitrification than those fed well water. This is reasonable since the surface water in this study, in general, had lower dissolved oxygen (DO) concentrations and higher bacterial concentrations than the well water. DO concentrations ranged from 1.8 to 2.5 mg l⁻¹ for surface water and 3.4 to 4.0 mg l⁻¹ for well water. As water passes through the BSF and remains inside until it is refilled again, the dissolved oxygen concentration in the water depletes. If the initial

Table 3 | p -values for ANOVAs comparing households using well or surface water for nitrate, nitrite and ammonia

Water quality parameter	p -values (95% confidence level)	
	Well water: comparison of all households using well water as their source	Surface water: comparison of all households using surface water as their source
NO ₂ ⁻ (mg l ⁻¹)	0.70	0.99
NO ₃ ⁻ (mg l ⁻¹)	0.11	0.38
NH ₃ (mg l ⁻¹)	0.98	0.22

Table 4 | Matched paired t-test results of untreated versus treated water

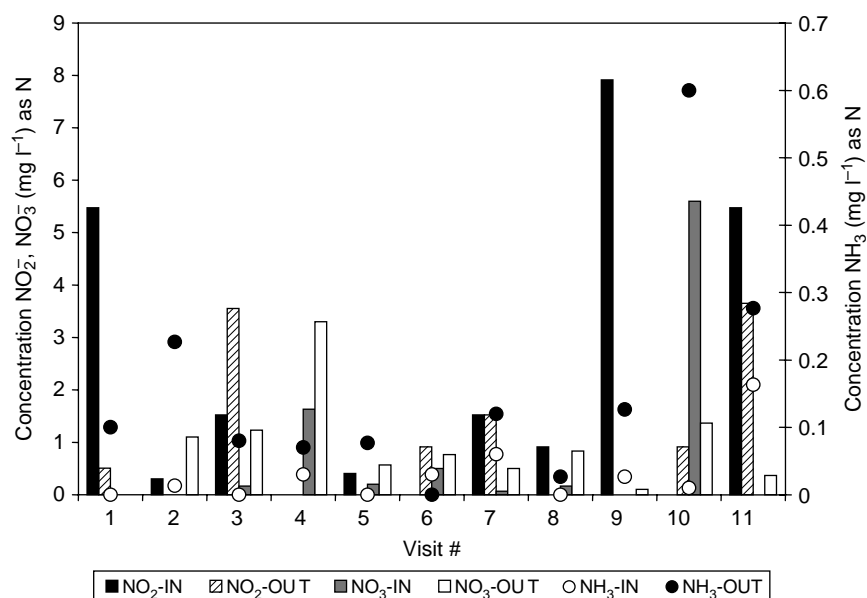
Water quality parameter	Well water (average change between untreated and treated water)	t-value (significance level)	Surface water (average change between untreated and treated water)	t-value (significance level)
NO ₂ ⁻ (mg l ⁻¹)	+0.4	11.51 (>99.9%)	+0.8	8.98 (>99.9%)
NO ₃ ⁻ (mg l ⁻¹)	+1.7	19.7 (>99.9%)	+1.1	13.67 (>99.9%)
NH ₃ (mg l ⁻¹)	-0.8	13.67 (>99.9%)	+0.1	25.4 (>99.9%)

concentration of DO is low at the outset, as is the case for the surface water, the water will likely become anoxic more quickly than in the case of well water. This low-oxygen environment is ideal for denitrification resulting in conversion of some of the nitrate to nitrite and ultimately to nitrogen gas (Belser 1979).

Another explanation for the increase in nitrite in treated surface water compared with well water is the fact that the oxidation process of nitrite to nitrate is more adversely impacted by environmental conditions than the oxidation of ammonia to nitrite. For instance, under high temperatures, the ammonia-nitrite process often dominates and results in increased nitrite concentrations. In general, water temperatures entering the BSFs ranged between 27 and 30°C; however, the BSFs in this study were often in direct sunlight and consequently temperatures over 30°C were observed in the treated waters from the filters. In addition, since pH governs the equilibrium between ammonia and

ammonium and nitrous acid and nitrite, the predominant oxidation processes (NH₃ to NO₂⁻; NO₂⁻ to NO₃⁻) are highly pH-dependent. In this study, pH in treated surface water ranged from 6.9 to 8.6, so at the higher pH levels the predominant oxidation process would have been ammonia oxidation; whereas at a lower pH both processes may occur simultaneously.

Additionally, an increase in ammonia was observed for filters treating surface water. An example of this is shown in Figure 9 where nitrate, nitrite and ammonia data for filter 'B15' are illustrated. The increase in ammonia concentration suggests that nitrate-ammonification is taking place inside the BSF. This is a process where nitrate is reduced to nitrite and further to ammonia and it occurs under the same conditions as denitrification (Belser 1979; Omnes *et al.* 1996; Cole 1996). Nitrate-ammonification is favourable in low oxygen environments, where the ratio of organic carbon to nitrate is high and where oxidized nitrogen compounds are

**Figure 9** | An example of nitrate, nitrite and ammonia data collected for filter 'B15'.

available for microorganisms to use as an electron acceptor (Omnes *et al.* 1996; Schaechter 2004). Enteric bacteria, specifically *E. coli*, have been found to reduce NO_3^- and NO_2^- under anaerobic conditions (Cole 1996; Schaechter 2004). When *E. coli* uses nitrate as an electron acceptor, high concentrations of nitrite build up inside its cytoplasm. As a result of this build-up, nitrite is rapidly ejected from the organism into the environment or further converted to ammonia by the enzymes present in the *E. coli*'s system (Cole 1996). The untreated surface water in this study contained high concentrations of *E. coli* bacteria as presented in Table 1. Consequently, it is plausible that under anaerobic conditions inside the BSF, ammonification may be occurring as a result of *E. coli* adapting to their environment and utilizing excess nitrate and nitrite as electron acceptors instead of oxygen. Ammonification can also be the result of the oxidation of organic nitrogen to ammonia. This occurs in an aerobic environment and could be occurring at the top of the BSF; therefore it is plausible that the resulting ammonia may have passed through the BSF and remained in the treated water. This would depend to a large extent on the filter flow rate and length of time the treated water remains in the filter before exiting via the spout.

From the results in Table 4, a *t*-test was performed to compare the effect of source water on the production or destruction of these compounds through the BSF. The results are illustrated in Table 5. They indicate that the variations in nitrate and ammonia concentrations differ significantly based on source water, while the variations in nitrite between source waters are only significant at the 70% confidence level.

Table 5 | *t*-test results for the difference between source waters in terms of nitrate, nitrite and ammonia

Water quality parameter	Is there a significant difference between source waters (well and surface water) in terms of increase/decrease of nitrogen compounds?	Significance level (<i>t</i> -test result of difference between well and surface water)
NO_2^- (mg l^{-1})	Yes, fairly significant	70%
NO_3^- (mg l^{-1})	Yes	95%
NH_3 (mg l^{-1})	Yes	>99.9%

Influence of flow rate and household practices on nitrite, nitrate and ammonia concentrations

The survey data collected from each household was compiled and correlated with the water quality results. These data included: filter flow rate documented at the time of visit and information regarding frequency of filling and amount of water filtered per day prior to the visit to the household. In the questionnaire, households were asked whether or not they had filled their filter that day already. If they had, they were asked how many buckets they poured into their filter at that time. The interviewer would document this information along with the size of bucket(s) the household used to fill their filter. In addition, if the household hadn't filled their filter yet that day, they were asked about the last time that they filled their filter. They were also questioned regarding how many and what kind of buckets they used to fill the filter. This information was compiled to make estimates of how long the 'pause time' would have been before the research team arrived to collect samples. The 'pause time' refers to the amount of time the water inside the BSF had been sitting before being filled again so a sample could be collected. Excel was used to create 'if statements' using the survey data collected. These statements were used to produce estimated pause times of 3, 4, 5, 6, 12 and 36 hours for each sample collected.

Household filter flow rate was correlated to nitrite, nitrate and ammonia concentrations in the treated water as well as the change in concentration from influent to effluent. There were no relationships found between household flow rate and the concentrations of these nitrogen compounds. Next, the pause times were correlated to these compounds as well as the change in concentration from influent to effluent. No significant relationships were found between pause time and changes in concentrations for nitrite, nitrate and ammonia. This was a surprising result, as one would expect that as the pause time increases, the concentration of dissolved oxygen would decrease. Consequently, this would increase denitrification potential and increase the formation of nitrite and decrease the concentration of nitrate. The following reasons may explain the lack of relationship in the current data. Recommendations are also provided for better examination of this issue.

- i. Samples were only taken every two weeks and consequently were probably too infrequent to properly correlate household practices to nitrification/denitrification processes inside the BSFs.
- ii. Data collected from the households weren't sufficiently detailed to determine the exact pause time in the filter. The results for this study were merely estimates. Much more household information would need to be documented to accurately determine the pause time. However, even with a more detailed questionnaire, households sometimes lie or forget information regarding their filter, making documenting this type of information challenging.
- iii. Daily visits over the course of a couple of weeks to individual households conducting questionnaires and collecting water samples may provide more insight into the influence of pause time on the changes in concentration of nitrates and nitrites in treated water from the BSF. However, one must be aware that the more frequently a household is visited, the more likely the household will change their practices to reflect what they think the researcher is looking for and may not be representative of 'actual' practices. This is always a challenge when conducting research with human subjects.
- iv. A controlled laboratory study using waters representative of field conditions may be useful in determining the effect of pause time on nitrification/denitrification processes inside the BSF. However, it should be noted that the results from the laboratory control experiments run in Cambodia for this study did not show the same results as observed in the field for nitrate, nitrite and ammonia. Two controls were conducted side by side at the RDIC laboratory, one with well water and the other with surface water. Samples were taken weekly and the filters were filled once daily at the same time each day. The results from these controls showed that nitrification was the dominant mechanism in the filters, as increases in nitrate and nitrite were evident each week. Consequently, laboratory studies may not be able to accurately represent what is occurring under field conditions. As a result, it is recommended that further research be conducted to investigate what differences there are between lab and

field operation and how these differences affect the performance of the BSF and nitrification/denitrification processes in the filter.

CONCLUSIONS/RECOMMENDATIONS

In conclusion, the findings indicated that simultaneous nitrification-denitrification processes were occurring inside BSFs under field conditions in rural Cambodia. As a result, the treated water samples from many of these filters did not meet WHO guidelines for nitrite and for combined nitrate-nitrite. Seventeen of 20 filters on average did not meet the $3.0 \text{ mg l}^{-1} \text{ NO}_2^-$ guideline and 17 also did not meet the combined nitrate-nitrite guideline ratio of 1. In the present study, denitrification seems more dominant in treated water from BSFs fed surface water than those fed well water. In addition, nitrate-ammonification seems to be occurring in some filters fed surface water causing increases in ammonia in treated water. It is hypothesized that filter operation and frequency of filling could be contributing to the formation of nitrite in treated water from these systems. However, more research would need to be conducted in order to confirm this hypothesis.

Until further research can be done, the following are a list of recommendations suggested for organizations looking to implement BSFs and for those who have already implemented them in the field:

- i. Source water quality should be tested prior to use in the BSFs. If filter implementation is to take place in a highly agricultural area with high fertilizer use or in an area where the presence of human and/or animal faecal contamination could be affecting water supplies, it is suggested that users use a water source that contains lower concentrations of nitrate and nitrite if possible.
- ii. Unless treated water is tested prior to being used and complies with guidelines, in order to reduce risk of blue baby syndrome, it is recommended that an alternative source of treated water be used to make formula for bottle-fed infants instead of relying on treated water from the BSFs.
- iii. Households who choose to boil their water after the BSF should be cautioned, as boiling could concentrate the nitrate and nitrite to more harmful levels.

iv. If concentrations of nitrite are high in treated water from the BSFs, the addition of chlorine or another oxidant may be useful to convert nitrite to the less harmful form of nitrate. This, however, will not always consistently reduce the combined nitrate-nitrite value to below the guideline value of 1. This will be largely dependent on how much nitrate and nitrite is initially present in the treated water. It will, however, help reduce the chronic and acute risk associated with high concentrations of nitrite in the water supply.

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