Effects of Boiling Water Temperature on Biofilm Formation in PTI Community Potable Water

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ABSTRACT

This study investigated the effects of boiling temperature and associated physico-chemical parameters on the Petroleum Training Institute potable water and the possibility of biofilm formation in its delivery systems. A total of 25 potable water samples were used for this study. The environmental parameters investigated were pH, conductivity, total dissolved solids (TDS), total suspended solid (TSS), dissolved oxygen (DO_1), / DO_5, salinity, resistivity, total coliform bacteria (as an indicator of possible biofilm presence in the distribution system) and biofilm . An overall prevalence of <1 of the total coliform bacteria was observed in the plus-boiling and minus-boiling potable water sample, except for the female hostel which showed moderate stain for the qualitative biofilm test. For the minus-boiling water sample, pH values were between 5.04±0.47 to 6.82±0.48; Total suspended solids ranged between 0.09±0.05-0.17±0.02, total dissolved solid ranged between 4.07±0.73 to 5.58±0.70, conductivity values ranged between 8.02±0.90 to 11.54±1.67, dissolved oxygen ranged between 1.97±0.26 to 3.12 ±0.13, the DO_5 ranged between 1.91±0.32 to 2.72± 0.29 while resistivity ranged between 7.79±0.13 to 10.88±0.18. Values for the Plus-boiling and filtered samples showed a pH range of 6.02±0.26 to 6.95±0.26; conductivity 7.21±0.10 to 9.88±0.67; DO ranged between 1.01±0.14 to 2.08±0.35, DO5 was 1.02±0.02 to 2.01±0.38, TSS and TDS ranged between 0.02±0.001, 3.74±0.62 to 0.03±0.002 and 4.95±0.42 respectively while resistivity ranged between 1.02±0.11 to 1.98±0.16. For all parameters analyzed, values obtained falls within the WHO limit for potable water except for the qualitative biofilm test on FSH minus-boiling water sample which gave moderate stain with 0.1% crystal violet stain and the pH values which fall below WHO acceptable limits. Boiling and filtration of potable water irrespective of the source is campaigned from this study in order to enhance the prevention and management of water borne diseases within the institute community.

Key words: Boiling temperature, Biofilm formation, Physico-chemical changes, and PTI community.

INTRODUCTION

The recently observed high *E.coli* levels in blood samples of the residents of the institute's hostel accommodation have been of great concern. Coliform bacteria are traditionally used as indicator of *E.coli* contamination of drinking water source. Although the detection of coliform may not pose serious effect but its presence suggests that there are other disease causing organisms present or that there may be the development of a layer of bacteria (biofilm) within the piping system amongst other causes. The primary reason why there is

concern for biofilm in drinking water is because of the growth of coliform bacteria in the pipe net work. Pontius (1995) reported the violation of drinking water standards in the USA in 1993, 1994 and 1995 following the growth of coliform bacteria in the distribution pipe net work. Studies have also shown that nearly 2000 systems in America are significant non compliers because of the repeated detection of coliform bacteria in finished drinking water. Recent studies have examined data from over 90 water systems to determine the factors that contribute to the occurrence of coliform bacterial in drinking water (lechevallier *et al*.,1996; Volk *et al.*, 1996). These

studies revealed that coliform occurrence in drinking water can be linked to the following factors: filtration, temperature, disinfectant type, residual assimilable organic carbon level, corrosion control and pipe material selection (Lechevallier *et al.*, 1996; Volk *et al.*, 1996). Biofilm can also be responsible for loss disinfectants residuals, increased bacterial levels, reduction of dissolved oxygen, taste and odor of drinking water, red or black water problems due to iron or sulfate-reducing bacteria, microbial influenced corrosion, hydraulic roughness and reduced material life (characklis and marshal, 1990). These effects of biofilm in principle depend on the physico-chemical conditions of the interface such as pH value, Oxygen concentration, redox potential and ionic strength etc. There are reports that the presence of biofilm can cause a change in the concentration of these parameters (www.efcweb.org). Research showed that systems that had cold water had increase in coliform occurrences. The influence of pH on purification capacity has generally been studied and in most cases pH has been reported to play a role in bacterial death (Hirn *et al.* , 1980; Tejordo *et al.*, 1992). Biofilm in drinking water pipe net work can be responsible for a wide range of water quality problems. The report that most of the persistent infections in humans, including the oral, lung, vaginal and foreign body-associated infections, are biofilm-based (Watnick and Kolter, 2000; Tumbarello *et al.*, 1996; Saify *et al.*, 2013) has also prompted our concern for this study. An important characteristic of biofilm is that individual microorganisms are held together by a polymeric substance excreted by the microorganisms, which forms an adhesive matrix (polysaccharide intracellular adhesion) that holds the biofilm together, allows it to attach to surfaces, and can serve as an encapsulation which protects the colony forming the biofilm (Ghellai *et al.*, 2014). This protective encapsulation is believed to play a role in some antibiotic-resistant infections experienced in recent times (Lewis, 2001).

This project was therefore conducted to investigate the effect of boiling temperature on possible biofilm formation in domestically stored potable water using coliform bacteria concentration as a marker. The result from this study will be used to inform consumers of water within the community on the significance of boiling and filtration in the prevention of common water born diseases.

MATERIALS AND METHODS

Collection and preparation of samples

A total of 25 representative samples were used for this study. 5 samples each from 5 taps chosen at random and located within female hostel, male hostel, senior staff quarters and Junior staff quarters and 5 150cl Eva water bought from different stores located on PTI road, Effurun. Samples were collected in the early hours of the morning from 20 taps selected at random. Samples were grouped into A, B, C, D and E. Samples in group A were 5 Eva bottled water (EVA), used as control. Samples in group B were potable drinking water collected from the noble female students hostel block (FSH). Samples in group C were water samples from 5 taps located in the male student hostel (MSH) while groups D and E were samples collected from the senior staff quarters(SSQ) and junior staff quarters (JSQ) respectively. Samples were analyzed immediately for physico-chemical parameters, biofilm and coliform test. A portion of the minus boiling water sample was incubated for 5 days for the determination of dissolved oxygen after 5days without any treatment for chlorine and other inhibitors of oxygen use up by microbes. The remaining samples classified as A_1, B_1, C_1, D_1 and E_1 were subjected to boiling, allowed to cool, filtered and analyzed for same physico-chemical parameters, qualitative biofilm and quantitative coliform test. The boiled and cool sample was also incubated for 5 days for the 5-day dissolve oxygen test.

Biofilm detection tube method

Dthe simple biofilm detection method described by Christensen *et al.*, 1982 with a slight modification was adopted. The sample suspected to contain test organisms was inoculated   in 10 mL of trypticase soy broth with 1% glucose (Marthur *et al.*, 2006) in test tubes. The tubes were incubated at 37ºC for 24 h. After incubation, tubes were decanted and washed four times with 200µl phosphate buffer saline (pH 7.2) and dried. Tubes were then stained with crystal violet (0.1%w/v (Boruki *et al.*, 2003; Mathur *et al.*, 2006). Excess stain was washed with deionized water. Tubes were dried in inverted position. The scoring for tube method was done according to the results of the control sample. Biofilm formation was considered positive when a noticeable film lined the wall and the bottom of the tube. The

amount of biofilm formed was scored in this present study as 1 for weak/none, 2 for moderate stain and 3 for high/strong. The experiment was carried out in triplicates and repeated thrice (Christensen *et al.,* 1982).

Determination of Coliform bacteria

Coliform bacterial concentration was determined using the multiple tube technique outlined Fernandez *et al.,* 1992. The media used for the detection of coliform in samples were the Lauryl tryptose broth, brilliant green lactose bile broth and eosin methylene blue for the presumptive, confirmatory and completed stages respectively. Samples were incubated at 35±0.5 ^{0}C for 24hrs±2hrs, 48hrs± 3hrs, 24hrs± 2hr and examined for gas formation in all three stages of coliform determination respectively. Coliform bacteria appraised were reported as CFU/100ml.

Determination of physicochemical properties

The physico-chemical parameters selected for this study were chosen in line with the multi barrier policy to assessing for drinking water of an acceptable quality which advices the monitoring of total coliform to be in conjuction with other indicators. pH of the water samples were determined prior to boiling and at the end of study duration before cutting of the base of plastic bottles. The pH of the water samples were determined using a pH meter digital model Jenway 3520 at 26 ± 2°C (AOAC, 2000). Conductivity, resistivity and salinity and TDS were determined using a potassium chloride calibrated multi seven mettler Toledo conductivity meter. Dissolved oxygen (DO) was analyzed according to the modified wrinkler and membrane probe method (first devised by wrinkler in 1888 and revised by USA EPA in their SOP, 2007) using Jenway 9500 dissolved oxygen meter before incubation at 20°C for day 5 DO analysis. Total suspended solids were analyzed gravimetrically according to the ASTMD 5907-10 for filterable and non filterable matter in water as outlined by Adeogun *et al.,* 2011.

Statistical analysis

Data were analyzed using the INSTAT® statistical soft ware. The non significant and significant differences were defined by 0.05<P<0.05 level of significance. Except for salinity values which

was constant for all samples analyzed, all results are expressed as mean±SD

RESULTS

Tables 3.1, 3.2 and 3.3 show the physico-chemical parameters and coliform count of non-boiling and plus-boiling potable water. For the plus- boiling drinking water, none of the 25 samples contained biofilm nor coliform while for minus-boiling potable water 0.99% of the 25 samples contained biofilm but no gas formation after subjection to coliform bacteria test.

The result of the qualitative analysis of biofilm presence in minus-boiling and plus -boiling potable drinking water is presented in Table 3.1.

The minus boiling FSH sample showed moderate stain in one of the five tubes tested for biofilm. Boiling of the same sample before test for biofilm showed no stain with 0.1% crystal violet (Table 3.1).

Table 3.2 represents the relationship coliform and physico- chemical parameters investigated in minus-boiling water drawn from 5 locations within the institute and its environs

Table 3.3 represents the relationship between coliform count and potable water samples drawn from 5 different locations with PTI community

Table 3.1: Qualitative Biofilm test for Minus-boiling and Plus-boiling potable water in PTI

		Qualitative Biofilm count (n=5)	
Grp	Sample	Minus-boiling	Plus-boiling
A	EVA	1	1
B	FSH	2	1
C	MSH	1	1
D	SSQ	1	1
E	JSQ	1	1

EVA= eva bottled water, FSH= female student hostel, MSH= male student hostel, SSQ= senior staff quarters, JSQ=junior staff quarters.

Table 3.2: Relationship between Coliform count and Physico-chemical parameters of Minus-boiling Potable water samples in PTI

Minus-boiling water samples(mean±SEM)n=5

Grp	Sample	Coliform (CFUs/100ml)	pH	Conductivity (µScm)	DO (mg/L)	DO5 (mg/L)	TSS (mg/L)	TDS (mg/L)	Salinity (PSU)	Resistivity (Ù.cm)
A	EVA	<1.0	6.82±0.48	8.02±0.90	3.12±0.13	2.72±0.29	0.09±0.05	4.07±0.73	0.02	7.79±0.13
B	FSH	<1.0	5.04±0.47	11.54±1.67	1.97±0.26	1.49±0.03	0.17±0.02	5.58±0.70	0.02	10.88±0.18
C	MSH	<1.0	5.16±0.27	10.11±1.38	2.58±0.43	1.91±0.32	0.16±0.03	5.08±0.71	0.02	9.56±0.21
D	SSQ	<1.0	5.98±0.24	10.88±1.12	2.55±0.17	2.07±0.04	0.15±0.05	5.10±0.70	0.02	9.87±0.16
E	JSQ	<1.0	5.84±0.52	10.86±0.13	2.76±0.68	2.26±0.52	0.12±0.36	5.06±0.72	0.02	9.76±0.25

EVA= eva bottled water, FSH= female student hostel, MSH= male student hostel, SSQ= senior staff quarters, JSQ=junior staff quarters.

Table 3.3: Relationship between coliform count and physico-chemical parameters of boiled potable water within PTI community

Plus-boiling water samples(mean±SEM)n=5

Grp	Sample	Coliform (CFUs/100ml)	pH	Conductivity (µScm)	DO (mg/L)	DO5 (mg/L)	TSS (mg/L)	TDS (mg/L)	Salinity (PSU)	Resistivity (Ù.cm)
A	EVA	<1.0	6.95±0.26	7.21±0.10	2.03±0.35	2.01±0.38	0.02±0.001	3.74±0.62	0.02	1.02±0.11
B	FSH	<1.0	6.05±0.38	9.88±0.67	1.01±0.14	1.02±0.02	0.03±0.002	4.95±0.42	0.02	1.98±0.16
C	MSH	<1.0	6.10±0.35	9.83±0.82	1.17±0.21	1.16±0.11	0.03±0.003	4.43±0.26	0.02	1.09±0.12
D	SSQ	<1.0	6.02±0.43	9.86±0.20	1.33±0.21	1.24±0.15	0.02±0.004	4.80±0.19	0.02	1.05±0.11
E	JSQ	<1.0	6.04±0.52	9.02±0.13	1.63±0.42	1.67±0.23	0.02±0.001	4.55±0.83	0.02	1.03±0.11

EVA= eva bottled water, FSH= female student hostel, MSH= male student hostel, SSQ= senior staff quarters, JSQ=junior staff quarters.

DISCUSSION

This study examined the effect of boiling temperature on possible biofilm formation and quality of potable water. pH is used as an indicator of how suitable water is for a specific purpose (Adeogun *et al.*, 2011; Jonnalagadda and Mhere, 2001). Examination of Table 3.2 reveals consistent low pH values. This could be due to the acidic nature of water from the Niger delta region of Nigeria following oil exploration and exploitation activities producing acid rain. This aligns with the work of (Efe and Mogborukor, 2012), who demonstrated the implication of acid rain from flaring of gasses during crude oil refining processes on the quality of water in the Niger delta. But when water samples were subjected to boiling, their pH values were enhanced although values obtained were not statistically significant (P>0.05). High dissolve oxygen concentrations are correlated with high productivity and little pollution, the observed reduction in DO concentration of minus-boiling water samples (Table 3.2) from the female hostel relative to their corresponding plus boiling (Table 3.3) water sample in this study could be an indication of pollution. A lthough, the value were not significant (P>0.05) and was within the WHO acceptable limits for potable water. The measurement of water conductivity is a typical way to monitor continuously the performance trend of water purification systems. Conductivity is the measure of water ability to pass electric current. Pure water is said to be a low conductor of electric current. The observed high concentration of electrical conductivity of minus-boiling potable water sample from the female hostel and all water sample analyzed relative to the control, Eva bottled water could also be an indication of contamination. Although, values obtained fall within WHO acceptable limit for potable water (2004). Conductivity is used as an estimation of total dissolved solids in water. The high levels of electrical conductivity observed in all samples of minus-boiling potable water relative to the control, Eva bottled water, could be due to the high levels of total dissolved solids of the same groups. Increase water temperature has been reported to reduce dissolve oxygen (DO) content of water. The low value obtained for DO of plus-boiling water relative to its minus-boiling water values could be due to boiling. Albeit, dissolved oxygen is not usually considered as a parameter for potable water analysis but in this study we investigated the DO value for day 1 and 5 to enable us ascertain that our suspicion regarding coliform bacteria presence especially in the FSH sample that showed a mild positive result in biofilm screening was incorrect. The dissolved oxygen values for boiled water, day1 and day 5 were almost equal. This could be that the minute biological agents in the minus boiling potable water sample was destroyed by the boiling process. Boiling also reduced the values obtained for total dissolved solids. This could imply that certain non filterable solute in the sample may have been precipitated out at boiling temperature. The low values recorded for total suspended solid in the plus-boiling potable water samples groups may possibly be because of the filteration carried out on the samples after boiling. In this study, salinity values were the same for minus boiling and plus boiling water samples irrespective of the source while resistivity value was reduced significantly (P<0.05) in plus boiling groups relative to the minus-boiling potable water samples. Except for pH values and the qualitative biofilm test result, values obtained in this study fall within the WHO standard for drinking water (2004).

CONCLUSION

From our findings, boiling of potable water within the institute is advocated in this study for the prevention and management of water borne diseases. Another interpretation of the result from this study could mean that the institute drinking water source may not be adequately treated since coliform can proliferate in storage system biofilm following the moderate biofilm presence observed in the minus-boiling female hostel water sample. One concern is whether opportunistic pathogens can proliferate.

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