Impact of Biofilms on Water Distribution System of a Tertiary Institution in Northern Nigeria

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Abstract

The aim of this research was to determine the impact of biofilms on water distribution system of Federal University Birnin Kebbi. Samples were collected from different water distribution pipelines in the University. Total heterotrophic count was carried out to determine the microbial load and Most Probable Method (MPN) was used to detect the presence of fecal coliforms in the water. Total heterotrophic bacterial count ranged between 1.6×10^3 to 3.9×10^3 cfu/ml. *Escherichia Coli* showed the highest frequency (25%) of occurrence, while the least frequency of occurrence (5%) was recorded for *Klebsiella spp* and *Enterobacter spp* respectively. The isolates identified were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella spp, Enterobacteria Spp, Salmonella Spp and Bacillus Spp.* Presence of these isolates is of significant concern and may cause some water borne diseases like diarrhea, dysentery etc. It is therefore recommended that water board treatment plant should use disinfectant chemicals like chlorine and perform regular proactive preventive maintenance, microbial monitoring and infrastructure replacement and repair so as to reduce the occurrence of biofilms in the Water Distribution system.

Keywords: Biofilms, Water distribution system, Microbiological water quality, Drinking water

1. Introduction

Water Distribution System (WDS) is an important part of water treatment that shows how drinking water is treated from the plant to the consumption points [1]. The distribution system carries along a number of microbial flora and complex organic matter, most of which are present in distribution systems in dissolved form, presented as Dissolved Organic Carbon (DOC) and monitored as Biodegradable Dissolved Organic matter [2]. No matter the degree in which water in the distribution system is treated, it is still not completely sterile. After the treatment processes some microbes can still survive and enter the distribution system through the pipe network in which they will attach themselves to the pipe wall and become part of biofilm [3]. Bacteria can exist in all types of water as they can adapt to most environmental conditions making the disinfection process very difficult and therefore could grow and attach themselves to the surface of the distribution system [4].

Biofilms are surface-associated, three-dimensional multicellular structures whose integrity depends upon the extracellular matrix produced by their constituent bacterial cells [5]. Biofilm formation occurs as a result of a sequence of events, adhesion of individual microbial cells to a surface, cell proliferation and aggregation into micro-colonies, matrix production, and cell detachment" [6]. The interaction of microbial cells with a surface and with each other initiates the process of biofilm formation, after which slimy extracellular polysaccharides and proteins are released

and biofilm subsequently matures [7-8]. Biofilms are network of interacting microbial communities at the solid-liquid interface and also at the liquid-gas interface that are attached to various substances [9]. Some disease - causing pathogens may survive in the biofilms but their survival time varies depending on the pathogens and distribution system that favors their growth while others cannot survive in it. However, biofilms can enhance the life of primary pathogens by protecting them from disinfectants [10]. These pathogens may be washed from the biofilm into the water column as a result of changes in the rate of water flow [11]. Thus, drinking water in the distribution system is not sterile, no matter the extent in which the water is treated. This means that microorganisms which are capable of surviving the treatment processes may be released to the community through the pipe network [12]. Utilization of biodegradable compounds which are either present in treated water or originate from materials in contact with drinking water facilitate the proliferation of micro-organisms in drinking water distribution systems. Studies have shown that biofilms in drinking water systems can serve as reservoirs for *Helicobacter pylori* (bacteria that can cause ulcers and cancer), *Legionellae* species (bacteria that can lead to legionellosis) and *Mycobacterium avium* (which can cause lung infections) [13].

Opportunistic bacterial pathogens like *Pseudomonas*, *Aeromonas*, *Klebsiella*, *Flavobacterium*, *Enterobacter*, *Citrobacter*, *Serratia*, *Acinetobacter*, *Proteus*, *Providencia*, *L. pneumophila*, *S. maltophilia*, and

Nontubercular mycobacteria (NTM) also served as reservoirs [14]. Fass *et al.* [15] demonstrated that a strain of *E. coli* takes a few minutes to contaminate the biofilm when introduced rapidly in a single experimental injection into a drinking water distribution pilot. There are standards used as reference or guide towards the production of any water for consumption. The World Health Organization (WHO) has recommended that water should be condemned if it is repeatedly found to contain one *Echerichia coli* per 100 ml [16]. This research was carried out with the aim of determining potential impacts of biofilms in Federal University Birnin Kebbi water distribution system.

2. Materials and Methods

2.1 Sample Collection
A total of 10 biofilm samples were collected at different drinking water distribution systems of Federal University Birnin Kebbi. To collect biofilms, the internal surface of the pipe was rigorously swapped using sterile swab sticks soaked in sterile distilled water. All the samples were transported under refrigerated conditions to the Microbiology Laboratory of Federal University Birnin Kebbi and analyzed immediately. The samples were labeled as WS 1 – 10.

2.2 Inoculation and Incubation
The samples were serially diluted by dipping the swab in sterile distilled water and shaken to ensure even distribution of organisms in the water and to make stock solution, using 9 test tubes each containing 9 ml of sterile distilled water. Using Micropipette, 1ml was taken from the stock solution and transferred into the first test tube and shaken, the procedure was then carried on up to the 9th test tube, and this procedure was repeated for the remaining 9 samples. An aliquot of 0.1ml was then pipetted from each dilution sample and plated on the respective nutrient agar using the spread plate technique. The plates were incubated at 37°C for 24 h. After incubation, plates with growths were observed and their colonies counted and reported as colony forming unit per mill (CFU/ML) [17]. All the procedures were carried out in duplicate.

2.3 Isolation and Sub-culturing
Depending on the types of colony observed on the primary culture plates, distinct colonies were sub-cultured on the nutrient agar plates by streaking method. The media were incubated for 24 h to obtain the pure cultures [17].

2.4 Identification and Characterization of the Organisms
The Gram staining was carried out as described by Cheesebrough [17]. Smear of inoculums from the isolates were prepared on grease free glass slide, the smear was then heat fixed and the gram staining was carried out. The biochemical tests were then carried out to characterize the isolates as described by Oyeleke and Manga [18] and Cheesebrough [17]. These included Indole test, Methyl Red (MR), Voges-Proskauer (VP), Urase, Catalase, Citrate, Coagulase, Triple sugar ion agar (TSI agar) medium, Hydrogen Sulphide, and Motility test

2.5 MPN Method
The procedure for testing water obtained from Federal University Birnin Kebbi water distribution system was done aseptically using MPN Method which was conducted in three steps: 1) Presumptive test 2) Confirmed test 3) Completed test.

i. Presumptive test:
MacConkey broth was used for lactose fermentation. The inverted Durham's tubes were used for the detection of gas formation by Gram negative coliform bacteria. Water samples (5 of 10 ml) were inoculated into each of 10ml of presumptive broth (double strength). 1 of 50ml water sample was added to a tube containing 50ml of presumptive broth (single-strength). After 48 h incubation at 37 °C, the number of positive tubes were recorded from each set and compared with standard chart to give presumptive coliform count per 100ml water sample.

ii. Confirmed Test:
In the confirmed test, positive samples from presumptive test were selected to determine the coliforms. Eosine Methylene Blue (EMB) media was used to differentiate *Escherichia coli* from Gram negative coliform bacteria by the production of greenish metallic sheen which confirms the presence of indicator bacteria *E. coli*. The production of color indication from colonies was observed after 24 h incubation at 37° C after streaking a loopful of sample from tube with positive growth.

iii. Completed Test:

The bacterial colonies on EMB media from confirmed test were inoculated in lactose broth at 44.5°C with Durham's tube and subculture the colony on Mac Conkey agar plate. Presence of fecal coliform indicator *E. coli* was confirmed by the production of gas and color changes in media [19].

3. Results and Discussion

3.1 Results
The findings showed that the water from pipeline sources was unsatisfactory for consumption as it contained potential pathogenic microorganisms including the indicator organism of fecal coliform. The result for total heterotrophic bacterial count is shown in Table 3.1. WS10 and WS3 were observed to have the highest bacterial count with 3.9×10^3 and 3.5×10^3 CFU/ml, respectively. WS1, WS6 and WS4 were observed to have the least bacterial count with 1.6×10^3, 1.8×10^3 and 1.9×10^3 CFU/ml, respectively.

Table 3.1: Total Heterotrophic Counts of Bacteria Isolates from Biofilms in FUBK Water Distribution System

S/N	Sample	Total Heterotrophic Count CFU/ml
1	WS1	1.6×10^3
2	WS2	2.1×10^3
3	WS3	3.5×10^3
4	WS4	1.9×10^3
5	WS5	2.3×10^3
6	WS6	1.8×10^3
7	WS7	3.0×10^3
8	WS8	3.2×10^3
9	WS9	2.0×10^3
10	WS10	3.9×10^3

The results of bacteria identified and percentage frequency of occurrence of each is presented in Table 3.2. *Escherichia coli* was found to have the highest occurrence, accounting for 25.0%, followed by *Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus ficalis* with 15.0%, while *Salmonella Spp* and *Bacillus Spp* had 10% each, *Klebsiella Spp* and *Enterobacter* had the least frequency of occurrence with 5.0%.

Table 3.2: Frequency of Occurrence of Bacteria Isolates from Biofilms in FUBK Water Distribution System.

S/N	Isolate	Number of Occurrences	Percentage of Occurrences (%)
1	*Staphylococcus aureus*	3	15.0
2	*Escherichia coli*	5	25.0
3	*Klebsiella*	1	5.0
4	*Salmonella spp*	3	10.0
5	*Pseudomonas aeruginosa*	3	15.0
6	*Enterococcus faecalis*	3	15.0
7	*Bacillus* spp	2	10.0
8	*Enterobacter* spp	1	5.0
	Total	**20**	**100**

Total coliform count was carried out using MPN method. The result of the presumptive coliform count is presented in Table 3.3. Four of the samples had a total coliform count ranging from 6 MPN/ 100 ml to 16 MPN/100 ml, while six (6) brands had no coliform contamination (<1 MPN / 100 ml).

Table 3.3: Total Coliform Count by MPN Method from Biofilms in FUBK Water Distribution System

Sample	Tubes with positive Rxn		MPN per 100ml		
	1*50ml	5*10ml		Upper	Lower
WS 1	0	0	<1	-	-
WS 2	1	3	9	2	21
WS 3	1	4	16	4	40
WS 4	1	2	6	1	15
WS 5	1	0	<1	-	-
WS 6	0	0	<1	-	-
WS 7	0	0	<1	-	-
WS 8	0	0	<1	-	-
WS 9	1	4	16	4	40
WS 10	0	0	<1	-	-

The results for confirmed test of MPN method are presented in Table 4. It was found that 2 out of 4 samples were contaminated with *E. coli*. The presence of indicator organism (*Escherichia coli)* isolates indicated fecal contamination as confirmed by the production of greenish metallic sheen on EMB.

Table 3.4: Confirmatory Test results from Biofilms in FUBK Water Distribution System

Sample	Growth on EMB	Production of green metallic sheen
WS 2	+	-
WS 3	+	+
WS 4	+	-
WS 9	+	+

3.2 Discussion

Bacteria can exist in all types of water as they can adapt to most environmental conditions making the disinfection process very difficult. They could grow and attach themselves to the surface of the distribution system [4]. It was noted that water distribution channels released the highest heterotrophic bacterial load (3.9 x 10³ cfu/ml) at WS10, while the lowest bacterial load (1.6 x 10³ cfu/ml) was recorded at WS 1. This is in line with the work of Abdullahi *et al.* [20] who reported the presence of fecal coliform counts of up to 1.4 x 10⁵ cfu/ml in water of Nsooba channel. Presence of high count provided sufficient evidence of the possible presence of pathogenic organisms from the poorly treated water being discharged into the channel [21]. The high incidence of pathogens recorded affirmed the call for increased rate of chemicals dose in water treatment plant.

In this study different microbial species were isolated from the water distribution system. The bacteria identified were *Staphylococcus aureus, Escherichia Coli, Klebsiella spp, Salmonella spp, Pseudomonas aeruginosa, Enterococcus faecalis, Bacillus cereus* and *Enterobacter aerogenes.* This is in line with the result of September *et al.* [22], who reported high numbers of *Pseudomonas, Klebsiella* and *Enterococcus spp.* from the biofilms of drinking water distribution systems in South Africa. Similarly, Akpor [23] reported the presence of *Escherichia coli, Salmonella Spp, Pseudomonas aeruginosa* and *Enterobacter Spp* from the biofilms of drinking water distribution systems. The study also showed the presence of fecal coliforms as the presence of *Escherichia coli* was confirmed. Presence of these isolates is of significant concern and may need further consideration. This may be as a result of pipe leakage, since soil is a natural reservoir of microorganisms and may find it favorable to thrive into the leakage place to form biofilms or due to poor treatment of the water from treatment plant. Furthermore, microbial pathogens can get washed into either drinking water supplies or receiving water bodies from animal and human fecal wastes [23].

The result obtained revealed enrichment in the diversity and population of microbial community in water distribution systems. Amongst the bacterial isolates, *Escherichia Coli* showed the highest frequency (25%) of occurrence and closely followed by *Staphylococcus aureus* (15%), *Pseudomonas aeruginosa* (15%), *Enterococcus faecalis* (15%), *Salmonella Spp* (10%), *Bacillus Spp* (10%), while the least frequency of occurrence (5%) was observed for *Klebsiella Sp* and *Enterobacter Spp* respectively. Presence of indicator organisms in the water such as *Escherichia Coli* is always used to determine the relative risk of occurrance of particular water borne diseases. This is in line with the finding of (Akpor, 2011) [23]. It is a fact that, contaminated water supplies are the source of several water borne diseases including, Cholera, Typhoid fever and Shigellosis [16].

It was also reported that density and diversity of these pathogenic microbes vary depending on the intensity and prevalence of the occurring infection [20].

4. Conclusion

The total heterotrophic bacterial count recorded ranged between 1.6×10³ to 3.9×10³. The bacteria identified were *Staphylococcus aureus, Escherichia Coli, Klebsiella Sp, Salmonella spp, Pseudomonas aeruginosa, Enterococcus faecalis, Bacillus cereus* and *Enterobacter aerogenes.* Amongst the bacterial isolates, *Escherichia Coli* showed the highest frequency (25%) of occurrence, while the least frequency of occurrence (5%) was recorded for *Klebsiella Spp* and *Enterobacter Spp* respectively. It is therefore recommended that management of water treatment plant should take important measures in reducing the number of microorganisms and reduce the leakages on the water distribution systems in order to deliver safe and portable drinking water.

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