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Effect of Lowering the pH of Sodium Hypochlorite on Control of *Pseudomonas aeruginosa* in Maintenance of Pharmaceutical Water System

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Abstract

Drinking water or potable water is a common source for generation of purified water or demineralized water which is used for processing of medicinal products such as tablets, capsules, syrups, and nasal sprays. Before allowing for purification, it is manufacturer's responsibility to ensure the microbial load control in the source water to get good quality product water and to avoid biofilm formation in waterlines. The current research involves the study of an effectiveness of sodium hypochlorite (NaOCl) at various concentrations of water pH on microbial control by following pour plate technique. The aim of this study was to examine the effect of lowering the pH on the recovery of *Pseudomonas aeruginosa*. Three groups were tested by challenging known population of *P. aeruginosa* by varying the pH of 5.0, 7.0 and 9.0. No significant difference was observed at pH 5.0 and 7.0 and the recovery increased when the pH increased at 9.0. Effective microbial control was obtained at neutral pH. The increase of pH resulted decrease of effectiveness of NaOCl.

Keywords: Biofilms, pH, Potable water, Purified water and sodium hypochlorite

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Introduction

Sodium hypochlorite (NaOCl) is a chemical substrate which is used for sanitization, cleaning and maintenance of water system, pipelines, and storage tanks.[1] This is also called as bleach and belongs to oxidiging agent family. NaOCl is slight greenish-yellow, or light yellow aqueous solution and is used to control the bio load and to prevent the formation of biofilms. NaOCl is popular today and has an extensive history in medicine and dentistry.[2] This chemicals also available in the form of anhydrous NaOCl, crystalline pentahydrate, gas, etc. When sodium hypochlorite dissolves in water, two substances are formed, which play a role in for oxidation and disinfection. These are hypochlorous acid (HOCl) and the less active hypochlorite ion (OCl-) HOCl and -OCl have been reported to react with a wide variety of biological molecules such as proteins, amino acids[3] lipids, and DNAat physiological pH conditions. The pH of the water determines how much HOCl is formed. To check the effectiveness of NaOCl at various types of water, it is proposed to initiate a study to check the microbial proliferation and inhibitory properties at various range of pH.[4]

Three types of water, namely, raw (source), potable, and purified water samples were collected for study. The source water from the various sources such as underground, municipal water or commercial supplied water which is being used from generations as potable water. The source water subjected to various types of pre-treatments which include filtration to remove macro and micro particles, chemical dosing for ensuring the required range of pH and other quality attributes, softeners to softening the water to decrease the hardness, and reverse osmosis to produce potable water. The outcome of RO is generally considered as potable water which is greater or equal to drinking water standards. Purified water is used in food industry and production of oral solid preparations and in other pharmaceutical applications, such as the cleaning of non-parenteral product-contact components and equipment.[5]

Gram-negative organisms are most common in water samples, especially *Pseudomonas aeruginosa* is a common contaminant in most of the pharmaceutical water systems and this produces biofilms. Infections with *P. aeruginosa* can be acquired from community settings (hot tubs, Jacuzzis, swimming pools), but occur mainly in healthcare settings, especially in critical care units and following procedures that involve physical breaches in host defenses, such as surgical incisions and the use of invasive devices. Populations at risk include neonates, patients with deep neutropenia, severely burned patients, patients with invasive devices (e.g., vascular and urinary catheters, endotracheal tube, and ventilator), and patients who have underlying pulmonary disease such as bronchiectasis and cystic fibrosis.[6] *P. aeruginosa* can cause a variety of infections, including pneumonia, bacteremia, urosepsis, and wound infections.[7] *P. aeruginosa* is a bacterium widely recovered from the environment that is capable of colonizing a number of wet and moist sites in plants and soils and a wide variety of aquatic environments.[8]

The study conducted at the pH range of 5–9 by collecting the samples at different intervals and different range of pH. Current efforts in microbial control using of the NaOCl revealed the scope of the study to ensure the outcome of microbial control dependency on pH factor. Hence, controlling of *P. aeruginosa* is an important step to maintain water quality. Basis to the above requirement, a

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study was performed to evaluate the effect of treated water pH on control of *P. aeruginosa*.

Materials and Methods

All the media and chemicals used for the present study were of HiMedia laboratories, India. NaOCl procured from Sigma Aldrich, USA.[9]

Sample Collection and Testing Plan

Three water different samples raw (source water), potable, and purified water were collected for 3 consecutive days in the month of September 2020 by following the aseptic conditions in a sterile container and stored at 2–8°C. The required media were prepared and sterilized at 121°C for 25 min.[10]

Sample Treatment

The water samples were treated with NaOCl to maintain chlorine content at 4 ppm. These samples were divided as three sets and adjusted the pH of each sample by adding 0.1 N HCl and 0.1 N NaOH.

Media Preparation

R2A agar media prepared using dehydrated media and sterilized at 121°C for 20 min for sterilization.[11]

Inoculum

Standard Microbial culture *P. aeruginosa* NCTC12924[12] selected for the study. The known concentration of culture (standardized inoculum) prepared to obtain final concentration of inoculum population as 50 cfu/ml and used for performing study.[13]

Inoculation

The standard culture of 0.1 inoculated to each Petri plate in duplicates and added R2A agar media and allowed for solidification. After addition of culture added approximately 20–25 ml of media by following pour plate technique to count, the number of colony-forming bacteria recovered from each type of sample subjected for study.

Positive Control and Negative Control

Simultaneous to test control performed a positive control by adding standard culture to serve as positive control. Negative control prepared by adding media to a sterile Petri plate.

Procedure

Total 4 sterile tubes used for the study, 1 tube containing 10 ml of sterile saline inoculated with *P. aeruginosa* to serve as positive control. Remaining three sample preparations performed for each type of sample to attain pH 5.0, pH 7.0, and pH 9.0 samples. The samples are subjected for testing by following pour plate technique for source water, potable water, and purified water. Each sample inoculated with a standardized inoculum by ensuring the concentration of inoculum should not more than 100 cfu/ml *P. aeruginosa* NCTC 12924 and allowed for 1-h contact time. After contact time, each type of sample has been plated 1 ml in a sterile Petri plates in duplicates and added 20–25 ml of R2A molten agar which is maintained at 45°C After adding media, plates were rotated clockwise and anticlockwise subjected for incubated at 30–35°C for 5 days.[14]

Results and Discussion

The test control samples shown variation of recovery at different pH and based on type of water. Three different samples did not show acceptable recovery at pH 5.0 and pH 7.0. The results of three days were observed in similar trend[15] (Tables 1 and 2). Based on the observations, it is noticed that the pH 5.0 & 7.0 controls the Pseudomonas aeruginosa, and recovery observed at pH 9.0 updated (Table 1) and the % of recovery observed calculated (Table 2). Also, the recovery percentages are increased in potable and source water respectively compared with purified water observed no change by increasing the pH from 5 to 6.[16]

Results of *P. aeruginosa* Recovered at Various pH Ranges

Table 1: The source water results at various pH levels

Day	*Type of water*	*pH 5.0 (cfu/ml)*			*pH 7.0 (cfu/ml)*			*pH 9.0 (cfu/ml)*		
		P 1	*P 2*	*Avg.*	*P 1*	*P 2*	*Avg.*	*P 1*	*P 2*	*Avg.*
Day 1	Source water	02	04	03	06	02	04	25	32	29
	Potable water	01	00	01	08	06	07	15	22	19
	Purified water	00	00	00	03	05	04	16	9	13
	Positive control	52	56	54	Standardcfu = 50/0.1 ml					
	Negative control	No growth			Not applicable					
Day 2	Source water	00	03	03	12	05	09	36	27	32
	Potable water	01	02	02	06	06	06	29	22	26
	Purified water	03	00	03	00	00	00	15	25	20
	Positive control	48	51	50	Standardcfu = 50/0.1 ml					
	Negative control	No growth			Not applicable					
Day 3	Source water	06	04	05	04	09	07	18	22	20
	Potable water	04	01	03	02	00	02	31	21	26
	Purified water	00	02	02	01	01	01	14	17	16
	Positive control	42	56	49	Standardcfu = 50/0.1 ml					
	Negative control	No growth			Not applicable					

P 1: Plate 1, P 2: Plate 2, Avg.: Average cfu of plate 1 and plate 2.

The percentage of recovery calculated using following formula.

$$\%\ of\ recovery\ observed = \frac{The\ Average\ cfu\ observed\ in\ sample\ control}{Positive\ control\ (Challenged\ cfu)} \times 100$$

% of Recovery Observed: % of Recovery Observed has been calculated by using above formula and observations are tabulated below.

Table 2: Test results

Day	*Type of water*	*pH 5.0*	*pH 7.0*	*pH 9.0*
Day 1	Source water	6%	7%	54%
	Potable water	2%	13%	35%
	Purified water	0	7	24
	Positive control	More than 70% recovery observed		
	Negative control	No growth observed		
Day 2	Source water	6	18	64
	Potable water	4	12	52
	Purified water	6	0	40
	Positive control	More than 70% recovery observed		
	Negative control	No growth observed		
Day 3	Source water	10	14	41
	Potable water	6	4	53
	Purified water	4	2	33
	Positive control	More than 70% recovery observed		
	Negative control	No growth observed		

Conclusion

Usage of NaOCl is a common practice to control microbial load present in water samples. Increase of bio load in source water or feed water for generation of purified water may lead to out of specification results, thus controlling bio load and biofilm formation is an essential step to control overall quality of water which is using for manufacturing of medicinal products. Thus, the present work reveals that the effectiveness of NaOCl at various pH ranges reveals that the lower pH is highly effective on the challenged microorganism. The increase of pH shown decrease of effectiveness of sanitizing agent. This is evident that the chemical which is using as a sanitizing agent is highly effective at lower pH and effectively controls the microbial load present in the samples.

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