Research Paper Microbiology

Effect of phosphorus in drinking water on cell counts of P. aeruginosa

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ABSTRACT

P. aeruginosa is the most dangerous human pathogen as it forms biofilms which makes it difficult to treat the infection with antibiotics. Thus presence of P. aeruginosa in drinking water is a reason of great concern. Nutrients in water especially phosphorus, even in low concentration, can promote extensive bacterial growth. This study presents a relationship between phosphorus concentration and growth of P. aeruginosa. A small change in phosphorus concentration below 5 ug/lit greatly decreased the growth of P. aeruginosa. Thus the growth of P. aeruginosa in drinking water can be controlled by lowering phosphorus concentration.

Keywords: Biofilms, P. aeruginosa, Phosphorus

Introduction

Water is one of the abundantly available substances in nature. It is an essential ingredient of animal and plant life. Good drinking water should not be harmful for health of consumers (1). Undesired health effects can be caused by chemical or microbiological agents found in some drinking waters (2). Microbes can be inactivated in the water plant by disinfection, but microbial growth begins when the residual content of disinfectants, often chlorine, disappears in drinking water distribution systems (3). Disinfection with high doses of chlorine is undesirable, since it can cause taste and odor problems in the drinking water. Therefore, microbial growth should be limited by eliminating their growth factors (3). Water treatment processes remove part of the chemical compounds and microbes from raw water, in treated water in water distribution system there still remain some microbes as well as the essential nutrients to support microbial growth (3). Microbial growth is predominantly affected by Assimilable Organic Carbon. However there are regions where Microbial growth is limited by Phosphorus. (4,5,6).

Some heterotrophic bacteria, like Pseudomonas Sp., Aeromonas, Bacillus, Klebsiella, and Acinetobacteria commonly found in drinking water may have virulence factors and thus must be viewed as potential health risks, particularly to immunocompromised consumers (7,8,9). These microbes probably do not multiply in the drinking water environment, but they may survive better in biofilms (10,11). Problems caused by the occurrence of biofilms are: bacteria are part of the food web and support the growth of higher organisms, they may generate turbidity, taste and odors, high counts of heterotrophic bacteria interfere with the detection of coliforms, microbes cause biocorrosion and they increase frictional resistances in the distribution system (12,13). Microbial nutrients can influence formation of biofilms. (12,14,15,16,17,18).

P aeruginosa is one of the most problematic human pathogens as it forms biofilms on infection. These biofilms are quite resistant to the actions of antibiotics and antibacterial drugs. This makes it difficult to treat these infections with antibiotics. This study focuses on role of phosphorus in limiting growth of P. aeruginosa.

Materials & Methods:-

Glasswares: - All the glasswares used were washed with 1 N HCl rinsed with deionized water and then sterilized in hot air oven at 250◦C for 10 hours.

Organism: - P.aeruginosa was used in the study. The bacterium was isolated from drinking water. It was cultured & stored in minimal salt solution at +4◦C.

Methods:

1. Effect of phosphorus concentration on growth of P. aeruginosa: Minimal salt solution was made in acid washed flask in deionized water. The salt solution contained (NH4)2 NO3-15 mg/lit, MgSO4.7H2O-0.6 mg/lit, CaCl2.2H2O-1.6 mg/lit, KCl-3.2 mg/lit, NaCl- 2.4 mg/lit and CH3COONa- 2mg/lit. A range of phosphorous concentration from 1 µg/lit -10 µg/lit was made with addition of different amounts of phosphorus (Na2HPO4) into 100 ml minimal salt solution. First flask contained 10 µg/lit of PO4-P. The concentration of phosphorus was decreased in each flask so that the last flask contained 1 µg/lit of PO4-P. Each flask was inoculated with 24 hours old broth culture of P. aeruginosa. All the flasks were incubated at room temperature to obtain maximum cell number. Bacterial cells were enumerated daily by spread plating on R2A agar. The plates were incubated at room temperature for 3 days before enumeration. The experiment was performed in triplicates.

2. Analysis of Phosphorus concentration in drinking water: Fraction of phosphorus supporting microbial growth in drinking waters of well, borehole and treated tap water was analyzed by a method described by Lehtola et al. (19)

Results:-

1. Effect of phosphorus concentration on growth of P. aeruginosa: Phosphorus concentration and growth of P. aeruginosa showed linear relationship. As the concentration of phosphorus decreased cell count also decreased (Figure 1). A small change in phosphorus concentration below 5 µg/lit greatly decreased the cell count of P. aeruginosa from 2.7566 X 103 CFU/ml to 0.9666 X 103 CFU/ml.

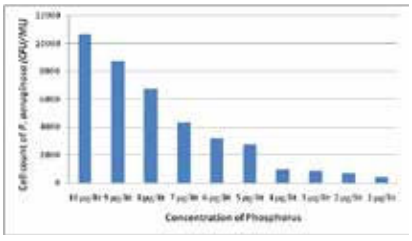

Figure 1: Effect of phosphorus concentration on cell count of P. aeruginosa.

Analysis of Phosphorus concentration in drinking water:
Concentration of phosphorus supporting microbial growth in drinking waters of borehole, well and treated tap water ranged from 2.5 µg/lit to 11.5 µg/lit. In borehole water, well water and treated tap water it was found to be 11.5 µg/lit, 7.5 µg/lit and 2.5 µg/lit respectively.

Discussion:-
Usually waterborne epidemics are caused by accidental contamination of drinking water eg. by flooding, surface runoffs or leakage of wastewater pipeline (20). Some heterotrophic bacteria particularly Pseudomonas sp. must be viewed as potential health risks when present in drinking water. It can metabolize a variety of organic compounds and can grow on very limited nutrient sources. In addition it is resistant to wide range of antibiotics and disinfectants (21). It is therefore desirable to remove Pseudomonas sp. from drinking water to minimize the risk to those who consume this water.

We present here a control measure to lower the number of P. aeruginosa in drinking water. We suggest lowering phosphorus concentration in water can limit the growth of P. aeruginosa. Our results showed that although phosphorus concentration upto 4 µg/lit promotes growth P. aeruginosa, lowering phosphorus concentration below 5 µg/lit can drastically decrease cell counts of P. aeruginosa. Amount of phosphorus supporting bacterial growth was also analysed here. Borehole water contained 11.5 µg/lit of phosphorus which was found to be maximum. This was followed by well water with 7.5 µg/lit and tap water with 2.5 µg/lit of phosphorus. This range was adequate, because in drinking water maximum microbial growth occurs in the range of 5 µg/lit to 10 µg/lit.(5,22).

Thus lowering phosphorus concentration in drinking water distribution system may help in reducing waterborne epidemics of P. aeruginosa infection. From the study it can be concluded that growth of P. aeruginosa in drinking water can be limited by lowering phosphorus concentration. Untreated ground water contains sufficiently high concentration of phosphorus to support bacterial growth. There is need to develop new water treatment processes that will efficiently remove the fraction of phosphorus from water which promotes bacterial growth.

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