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Research Article

REMOVAL OF PATHOGENIC BACTERIA IN HOSPITAL WASTE WATER USING CHITOSAN

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ABSTRACT

Elimination of pathogenic microorganism from waste water is a matter of concern in last few decades. Untreated waste water discharged from hospitals, produce health hazards to the living organisms. In this study, chitosan was used to eliminate pathogenic bacteria from hospital waste water. Uncountable colonies of *E.coli* (more than 300 colonies) and countable colonies (22 colonies) of *Samonella* were observed in the hospital waste water. After the antibacterial treatment period of 24 hours, pour plate method illustrated that, the *E. coli*, S. *bongori* and S. *enterica* were eliminated from the hospital waste water.

INTRODUCTION

Untreated waste water discharged from hospitals, produces health hazards to the living organisms. Various methods have been employed to eradicate microorganisms in hospital waste water before discharge. They are expensive and having demerits also. Generally, iron-based coagulants, aluminum hydroxide or polyaluminium chloride (PACl) have been used to remove the microorganisms. However, site-specific water-quality conditions like level and type of natural organic matter, pH, temperature, alkalinity and turbidity had a greater effect on removal efficiencies than the type of coagulant (Yongabi, 2010). The self-purification processes using biomaterial is the best known method, which effectively removes the bacteria and improves water quality. Among the biomaterials, chitosan is a versatile biomaterial, have attracted considerably due to their antimicrobial and antifungal activities (Jung *et al*., 1999).

Chitosan is a wealth from waste. It is the deacetylated derivative of chitin. Chitin is a versatile biopolymer present in the exoskeleton of crustaceans which can be obtained from the shell waste of the crab, shrimp processing industries. It is also found in a wide range of natural sources, such as fungi, yeast and insects. This polysaccharide is a partially deacetylated co-polymer of β-1, 4 linked glucosamine and N-acetyl glucosamine. It is said to be the second most abundant natural biopolymer on earth next to cellulose (Usui *et al*., 2004).

Biocompatibility, biodegradability and non-toxicity of chitosan have attracted much scientific and industrial interest in the fields of biotechnology, pharmaceutics, wastewater treatment, cosmetics, agriculture, food science, and textiles (Zheng *et al*., 2000 and Jeon *et al*., 2001). Furthermore antimicrobial activities of chitosan derivatives have received considerable attention in recent years due to the problems associated with chemical fungicide agents. In this study, mechanically stable chitosan beads were prepared and were used to eliminate pathogenic bacteria from hospital waste water.

MATERIALS AND METHODS

Five liters of untreated hospital waste water was collected from K. K Orthopaedic Hospital at parvathipuram, Nagercoil and Five hundred ml of hospital waste water was poured into a sterile conical flask under aseptic condition and was sealed by sterile cotton plug for 24 hours (control flask). For the enumeration and isolation of pathogenic bacteria from untreated waste water sample, Serial Dilution Technique was

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followed. Using separate fresh sterilized pipettes 0.1ml of sample from each dilution test tube (10-1, 10-2, 10-3 and 10-4) was transferred into each agar plates respectively (10-1, 10-2, 10-3 and 10-4). Uniformly spreaded the sample on the agar plate by 'L' rod and the lids were placed over the agar plate and incubated at 30 0 c for 48 hours.

Antibacterial treatment

Prepared chitosan beads were arranged in sterilized steel rods and placed inside a sterile conical flask (experimental flask). Transfer 500ml of Hospital waste water into the experimental flask under aseptic condition and was sealed by sterile cotton plug for 24 hours. 0.1ml of treated sample in experimental flask was spreaded on the SS agar plate by spread plate technique. Incubate the plate at room temperature for 48 hours.

Table1 Number of bacterial colonies in the control sample culture

SL.No	Dilution factor	Bacterial colonies			
		Total	E.Coli	Salmonella	Shigella
1	Dilution 10^{-1}	<300	high	22	0
2	Dilution 10^{-2}	252	249	3	0
3	Dilution 10^{-3}	45	42	3	0
4	Dilution 10^{-4}	3	3	0	0

Table 2 Number of bacterial cells in the control sample culture

SL.No	Dilution factor	Bacterial cells/ml			
		Total	E.Coli	Salmonella	Shigella
1	Dilution 10^{-1}	<3000	high	220	0
2	Dilution 10^{-2}	25200	24900	300	0
3	Dilution 10^{-3}	45000	42000	3000	0
4	Dilution 10^{-4}	30000	30000	0	0

Plate1

Chitosan Beads

Control Flask

Treatment Flask

Treated Sample Culture

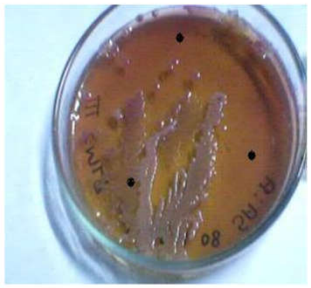
Plate 2 Untreated Sample Culture

Dilution 10^{-1}

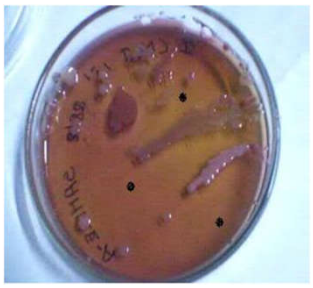
Pink coloured colony=E.Coli and

Black coloured colony=Salmonella

Dilution 10^{-2}



Dilution 10^{-3}



Dilution 10^{-4}

RESULTS

In control sample, uncountable bacterial colonies were observed in the dilution10^{-1} and countable bacterial colonies (<300 colonies) were observed from the dilution10^{-2} to 10^{-4}.

In the dilution 10^{-1}, more than 300 bacterial colonies (3000 bacteria /ml) were observed. Of which, E.Coli colonies were high in count and salmonella colonies were 22 (220 bacteria/ml) in count.

In the dilution 10^{-2}, 252 bacterial colonies (25200 bacteria /ml) were observed. Of which, E.Coli colonies were 249 (24900 /ml) and salmonella colonies were 3 (300 bacteria /ml) in count.

In the dilution 10^{-3}, 45 bacterial colonies (45000 bacteria /ml) were observed. Of which, E.Coli colonies were 42 (42000 /ml) and salmonella colonies were 3 (3000 bacteria /ml) in count.

In the dilution 10^{-4}, 3 bacterial colonies (30000 bacteria /ml) were observed. Of which, E.Coli colonies were 3 (30000 /ml) in count and salmonella colonies were not observed.

There is no bacterial growth in the chitosan treated waste water sample.

DISCUSSION

Common pathogenic gram-negative bacteria in hospital waste water are, E. coli, Salmonella and shigella. So the selective media (SS agar) for and shigella being used to enumerate these bacterial colonies. In SS agar media, E.coli appeared as pink coloured colonies, Salmonella appeared as black coloured colonies and shigella appeared as colourless colonies. Hospital waste water sample collected for this study was free from sigella. So, there is no colourless colony. But, uncountable colonies of E.coli (more than 300 colonies) and countable colonies (22 colonies) of Samonella were observed. Serial dilution technique is employed to count the E.coli colonies.

Chitosan has the innate characteristic of antimicrobial activity itself (Darmadji *et al*, 1994). Chitosan is a natural biocompatible cationic polysaccharide and also a weak polyelectrolyte, whose antibacterial activity has received considerable attention in recent years. Hence, to give mechanical strength and stability to chitosan up to the period of complete antibacterial treatment, it was prepared in the form of beads and neutralized with sodium hydroxide.

After the antibacterial treatment period of 24 hours, pour plate method illustrated that, both the E. coli and Salmonella were eliminated from the hospital waste water. The exact mechanism of antibacterial action of chitosan is still unknown, but different mechanisms have been proposed. Interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents (Fu *et al*., 2005). The mechanism of chitosan antibacterial action involves ionic interaction between the chitosan and the bacterial surface that changes the membrane permeability also caused leakage of glucose and lactate dehydrogenase from E. coli cells (Tsai *et al*., 1999). Chitosan affected growth and haemolysin production of Aeromonas hydrophila (Taha *et al*., 2002). Electron microscopic observation indicated that the surface of the bacteria was expanded, distorted when it was treated with chitosan. Chitosan could cause leakage of cell contents of the bacteria and disrupt the cell wall (Moon *et al*., 2007).

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