REMOVAL OF *ESCHERICHIA COLI* FROM WATER BY SYSTEM BASED ON PHOSPHORUS - CONTAINING SYNTHETIC PREFORMED POLYMER

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ABSTRACT

A study of the removal of Escherichia Coli cells from water by α-hydroxyphosphonic group grafted on styrene-6.7% divinylbenzene copolymer was made. The α-hydroxyphosphonic - containing synthetic preformed polymer is well suited for subsequent use of the product as antibacterial agent.

INTRODUCTION

Contamination by microorganisms is of great concern in a variety of areas, such as medical devices, healthcare products, water purification systems, hospitals, dental office equipment, food packaging, food storage, household sanitation, etc.[1, 2]

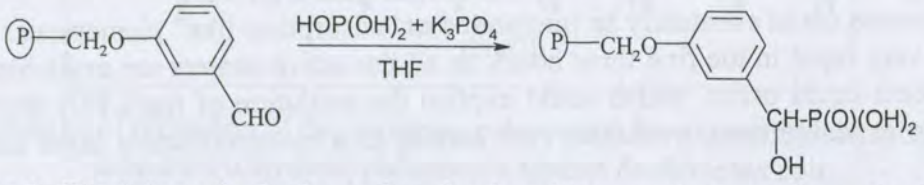
There is a definite need for new materials with antimicrobial activities. An area of polymer research that presents great current interest, yet has received insufficient attention, is that of the development of polymers with antimicrobial activities, generally known as polymeric biocides. The problem of the preparation of polymeric biocides can be solved in many cases, if the bactericide is covalently grafted on polymeric carriers or other insoluble support materials [3-5]. Since the early 90's α-hydroxyphosphonates have attracted much attention due to their wide ranging biological activity [6-8] and their usefulness as synthetic intermediates for other biologically important α-substituted phosphoryl compounds.

This study is concerned with the removal of *E. coli* cells from water by polymeric biocide derived from the polymer-analogous reaction between phosphorous acid and aldehyde grafted on styrene -6.7% divinylbenzene copolymer.

MATERIALS and METHODS

1. Phosphorus - containing synthetic preformed polymer

The obtaining of the α-hydroxyphosphonic acids by reaction between phosphorous acid and aldehyde grafted on styrene -6.7% divinylbenzene copolymer (Scheme 1) was confirmed by IR spectrum, the group P =O was identified.



Scheme 1. Obtaining of α-hydroxyphosphonic acid on synthetic preformed polymer.

2. Antibacterial activity study of the polymer-grafted α-hydroxyphosphonic group against Escherichia coli

The antibacterial activity of the polymer-grafted α-hydroxyphosphonic group against *Escherichia colis* was studied in batch system.

In an Erlenmayer flask fitted with magnetical stirring were introduced 0.6 g of polymeric disinfectant, 29 mL of sterilized water and the mixture was stirred at room temperature for 1 hour.

1 mL culture of *E. coli* (contained 10^7 cells/mL) was then added in the mixture containing the polymeric disinfectant, at room temperature and stirring was continued. At a precisely interval samples of 1 mL were collected and the number of colony forming units (C.F.U) was determined by the decimal serial dilutions method. From each diluted solution two Petri plates were seeded with 1 mL of solution, then 10 mL of nutrient gelose melted and cooled at 45 °C was added and after homogenisation by stirring, the mixture was left to solidify at room temperature. The obtained plates were incubated for 24 hours at 37 °C. The number of colonies/mL was determined as an average value for the two plates by standard procedures [9].

RESULTS

In Figure 1, is presented the process of decrease of the log (CFU) as a function of the exposure time.

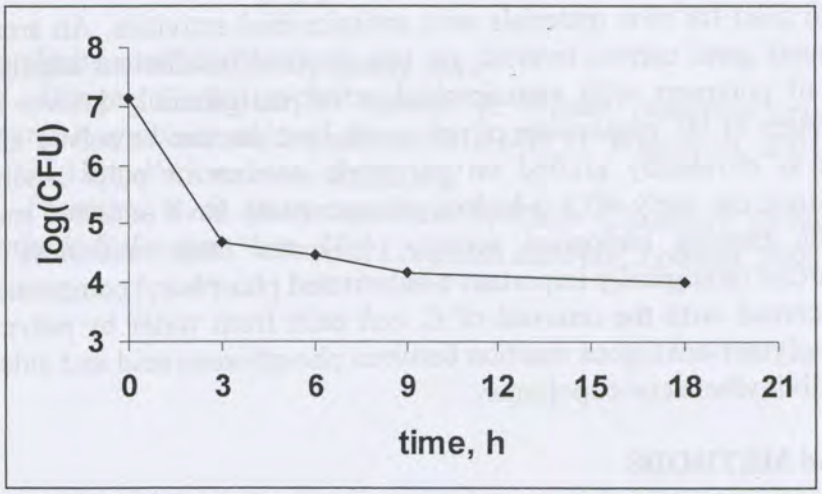


Fig.1. Plot of log(CFU) versus the exposure time using polymer-supported α-hydroxyphosphonic group against *E. coli*.

In order to compare quantitatively the ability to decrease the viable cell number, the following was considered. In the early stage of contact, a linear relation was observed between the logarithm of viable cell number and contact time until 3h.

This process could eventually be interpreted as "adsorption-like" phenomena. Initially, the process is very rapid in the first three hours, as all the active centers are available. Then a saturation process could occur, which could explain the evolution of log(CFU) depend as a function of the exposure time, evolution very similar to a monomolecular layer adsorption isotherm.

Linear correlations were observed until about 3 h from the start. Thus the removal coefficient of viable cell number (R) Kao et al.'s [10], Isquith and McCollum's and Kawabata et al.'s [11] was defined as equation (1) and calculated:

$$C_R = \frac{V}{W \cdot t} \cdot \log \frac{N_0}{N_t} \qquad \text{(ml / g·h)} \qquad (1)$$

where: V is the volume (mL) of viable cell suspension, W- the dry weight (g) of the polymer, t- the contact time (h), N_0 the initial viable cell number and N_t the viable cell number (cells/mL) at contact time t.

Table 1 lists removal coefficient value of viable cell numbers from water by α-hydroxyphosphonic group grafted on copolymer.

Table 1. Removal coefficient C_R[a] of viable cell numbers of *E.coli* from water at contact with the polymer.

Prod.	N_0[b] (cells/mL)	N_t[c] (cells/mL)	C_R (mL/g·h)
St-6.7%DVBHOPHOS	$13.5 \cdot 10^6$	$0.49 \cdot 10^5$	40.66

[a.] Determined at 37°C by the contact of the insoluble polymer (0.6 gram) with 30 mL of viable *E. coli* cell suspension.
[b.] N_0 – the initial viable cell number. [c.] N_t –the viable cell number at contact time (t = 3 hours).

The antibacterial activity of was evaluated through the percentage of reduction of the colony units. The percentage of reduction of the colony units (R, %) was determined from the decimal logarithm of the colony forming units number (CFU). According to literature date [12] a species is considered as having bactericide action if 99.9% of the bacteria cells are destroyed in 18 hours.

As after 18 hours of exposure, around half of the bacterial cells were destroyed; we can characterize the product as bacteriostatic (R = 43.90 %). The resulted is presented in Figure 2.

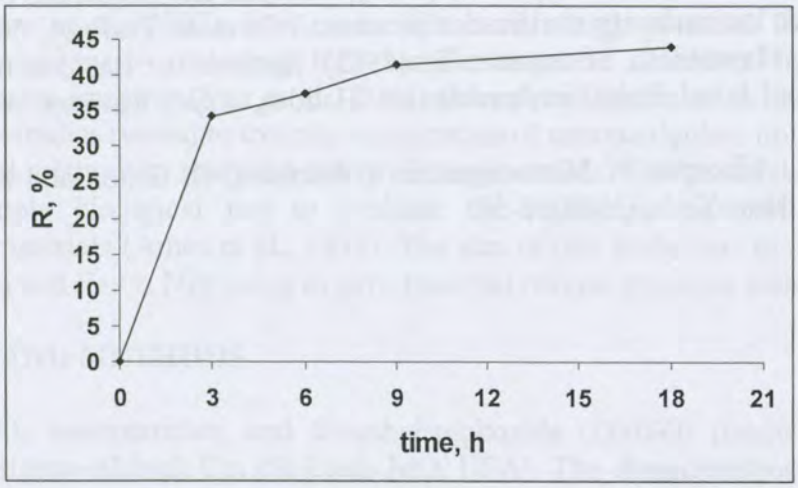


Fig. 2. Percentage of reduction of the surviving cells versus the exposure time using polymer-supported α-hydroxyphosphonic against *Escherichia coli*.

CONCLUSIONS

The removal of *E. coli* cells from water by α-hydroxyphosphonic group grafted on styrene-6.7% divinylbenzene copolymer was made. Removal coefficient value of viable cell numbers from water is 40.66 mL/g·h. As after 18 hours of exposure, around half of the bacterial cells were destroyed; the product is bacteriostatic (R = 43.90 %).

LIST OF REFERENCES

[1] Patel M. B., Patel S. A., Ray A., Patel R. M. (2003). Synthesis, Characterization, and Antimicrobial Activity of Acrylic Copolymers, *J. Appl. Polym. Sci.*, 89, p. 895-900.
[2] Park E.-S., Lee H.-J., Park H.-Y. et all, (2001). Antifungal effect of carbendazim supported on poly(ethylene-*co*-vinyl alcohol) and epoxy resin, *J. Appl. Polym. Sci.*, 80, p. 728-736.
[3] Ikeda T., Hirayama H., Yamaguchi H., et all, (1986). Polycationic Biocides with Pendant Active Groups: Molecular Weight Dependence of Antibacterial Activity, *Antimicrob. Agents Chemother.*, 30, p. 132-140.
[4] Ikeda T., Yamaguchi H. Tazuke S., (1990). Molecular Weight Dependence of Antibacterial Activity in Cationic Disinfectants, *J. Bioact. Compat. Polym.*, 5, p. 31-42.
[5] Tashiro T., (1993). Removal of *Escherichia coli* from water by systems based on insoluble polystyrene–poly(ethylene glycol)s, –polyethylenimines, and –polyethylenepolyamines quaternized, *Journal of Applied Polymer Science*, 43, p. 1369-1377.
[6] Stowasser B., Budt K.-H., Jian-Qi L., et all, (1992). New hybrid transition state analog inhibitors of HIV protease with peripheric C_2-symmetry, *Tetrahedron Lett.*, 33(44), p. 6625-6628.
[7] Maier L., (1993). A novel synthesis of bis-(α-hydroxyalkyl)phosphinic acids, *Phosphors, Sulfur and Silicon,* 76, p. 119-122.
[8] Rath N.P, Spilling C.D, (1994). The enantioselective addition of dialkylphosphites to aldehydes: Catalysis by a lanthanum binaphthoxide complex, *Tetrahedron Lett.*, 35(2), p. 227-230.
[9] Raducanescu H., Bica-Popii V., (1986). *Bacteriologie Veterinara*, Ed. Ceres, Bucuresti.
[10] Kao I.C., Robker D.E., Fan L.T., et all, (1972), Analysis and properties of a quaternary ammonium triiodide ion exchange sterilization process, *J. Ferment. Technol.,* 50, p. 438-445.
[11] Kawabata N., Hayashi T., Matsumoto T., (1983). Removal of Bacteria from Water by Adhesion to Cross-Linked Poly(Vinylpyridinium Halide), *Appl. Environ. Microbiol.*, 46, p.203-210.
[12] Daniels S. L., Adsorptio of Microorganism to Surfaces, G. Gitton and K.C. Marshall. (Eds), John Wiley, New York, (1980), 2-58.