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Antimicrobial properties of iodine based products

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IO_3^- (100 ppm +) in water exhibits antimicrobial properties when taken alone or with salt. Polyiodide resin killed experimental bacteria on contact and exhibited excellent antimicrobial properties while resin loaded with IO_3^- was not effective. Therefore, polyiodide resin would be an efficient system for water disinfection.

Keywords: Antimicrobial properties, Iodate, Iodated resin, Iodinated resin, Oral rehydration, Water disinfection

Introduction

Iodine and iodinated resin have been evaluated for water treatment[1]. Iodine (2.5-7.0 mg/l water) has been used in water treatment since 1900, especially for troops[2-6]. Iodine's solubility in water depends on temperature[7,8] (0.29 g/1 at 20°C, 0.78 g/l at 50°C and 4.45 g/1 at 100°C). Its hydrolysis and hence speciation in water depends on pH and concentration of titrable iodine in solution[6]. Taylor & Butler[9] found iodine most effective against poliovirus at pH 9.0. Karalekas *et al*[10] found little difference in bactericidal effects of 1.0 mg/1 iodine at pH 5 and 7, but a pronounced reduction in effect at pH 9.0. Elemental iodine (I_2) is reported[11] more effective (3-6 times) against spores than hypoiodous acid (HIO), an effective bactericide and virucide. Chang[12] found I_2 more effective (2-3 times) than HIO against cysts of *Entamoeba histolytica*, whereas HIO to be 40 times more effective against viruses than I_2. Clark *et al*[13] studied vulnerability of viruses to HIO. Chang[12] found HIO to be 3-4 times more effective than I_2 against *Escherichia coli*. Iodine inactivates faecal bacteria in poor quality water at different turbidities[14,15]. It provides disinfection of water across a wide range of pH[6,16] due to presence of various iodine moieties.

A single serving of many seafoods provide more iodine than daily intake from water[17]. Also, long-term ingestion of iodated water[16,18] failed to elicit any harmful effects. On the other hand, in goiter prone areas, daily intake of iodized salt (I^- or IO_3^-) is recommended as follows[19]: adults, 150; pregnant women, 220; and breastfeeding women, 290 µg. Study[20] is available on the amount of I^- and IO_3^- in commercial iodized salt samples. It is of interest to see if IO_3^-, present in salt being administered for oral rehydration[21], would be effective in water disinfection.

A strong basic quaternary ammonium- anion exchange resin saturated with tri- or penta-iodide can transfer I_2 to living cells by direct contact[22], effectively killing several bacteria, protozoa and viruses[22-25]. Stability, availability of halogen on a demand-release basis and prolonged (>7 y) effectiveness are additional characteristics reported for iodinated resins as effective water disinfectants[22,23]. Bactericidal activities of tri- and penta-iodinated resins as disinfectants for potable quality water containing *Legionella pneumophila* has been evaluated[26]. Positively charged, quaternary ammonium, strong base, polystyrene resin beads containing electrostatically held triiodide and pentaiodide anions, have been used in microbial check valve (MCV) as potable water disinfectants[27-29]. Effect of microgravity on *E. coli* and MS-2 Bacteriophage disinfection by iodinated resin has been studied[30,31]. Pentaiodide resin column has detrimental effect on oocyts of *Crytosporidium parvum*[32]. An iodinated copolymer was evaluated for water disinfection[33] and also a polymeric hydrogel device loaded with I_2 was evaluated for sustained release of iodine for water disinfection[34]. Common method of studying effectiveness of an antibacterial agent is to see its effect on faecal coliform (FC) as indicator bacteria. Level of agreement between

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MPN for FC and H_2S strip test is good but could depend on water source[35].

This study presents antimicrobial properties of IO_3^- with and without NaCl at concentrations taken in iodized salt for various applications. Also, iodine was immobilized as IO_3^- and polyanionic forms on to anion exchange resins and evaluated for antimicrobial properties.

Experimental

Two types of contaminated water samples [sludge from a sewage treatment plant (STP) at Okhla, Delhi, and *E. coli* B-19 strain suspended in water] were taken for testing efficacy of antimicrobial agent. Inoculum, taken from Department of Biochemical Engineering and Biotechnology (DBEB), IIT, Delhi, was grown overnight in Luria Broth (Himedia) in Erlenmeyer flask (250 ml) at 37°C in an incubator and kept in a refrigerator. Typical distilled water suspension of inoculum was taken as stock that contained *E. coli* B-19 strain (2.5 x 10^8 CFU/ ml).

Evaluation of Antimicrobial Properties of Iodate and Iodized Salt

Using sterile distilled water, solutions of KIO_3, pure NaCl and iodized salt and combinations of NaCl and KIO_3 were made for testing antimicrobial properties. KIO_3 and NaCl were procured from Merck, India. Iodinated salt was commercially available from market. Salt contained iodate (40 µg/g) as estimated by iodometric titration[36].

Contaminated water samples were added to different concentrations of IO_3^- (1000, 500,100, 50, 20, 10, and 1 ppm), iodized commercial NaCl salt (1000, 500 ppm), NaCl pure (5000, 1000, 500 ppm) and a combination of NaCl pure (1000, 500 ppm) with IO_3^- (varied over 500, 100, 50, 20, 10, 1 ppm). Calculated amounts of contaminated water sample was added to keep concentration of salts at indicated level while having bacterial counts at desired level. Samples were analyzed immediately after addition (t=0) of inoculum and then after keeping for 24 h, to check drop in bacterial counts. Samples were analyzed by standard MPN tests[37] for total coliform (TC), FC and by H_2S test[38]. MPN test was performed by nine multiple tube dilution technique using double and single strength MacConkey broth for *E. coli*. TC test was performed by using BGLB (Brilliant Green Lactose Bile) broth at 35°C and FC test using EC media at 44.5°C. MPN positive water samples were further inoculated for thermo-tolerant FC at 44.5°C for 24 h in EC medium and MPN positive tubes were tested for TC in BGLB medium. Results were recorded as gas in BGLB and EC media. MPN test[37] results are reported as MPN index/100 ml.

Paper strips[38] for H_2S strip test were prepared using Manja's medium. Medium (1 ml) was added to each screw cap bottle (20 ml) with folded paper strips and sterilized at 121°C for 15 min. Test sample (20 ml) was added to each bottle for testing its bacteriological quality in duplicate. Bottles were incubated at RT for 24 and 48 h. Positive (+) H_2S strip test and hence contamination of water samples was indicated by change in colour of medium to black. Test was considered negative (-) when no colour change was seen in medium.

Evaluation of Antimicrobial Properties of Iodated and Iodinated Resin

Bacterial concentration was determined by plating appropriate dilutions on MacConkey agar plate and incubating at 37°C. Stock solution was diluted to give samples with required level of bacterial counts. Iodinated resin (iodine loaded as polyanions) was prepared from a strongly basic, quaternary ammonium (polystyrene) type, anion exchange resin (Amberlite, IR 400, Merck, India). Resin was iodinated in laboratory using reported[39] procedure equilibrating resin with excess of iodine dissolved in KI solution. Amount of iodine (1.1 g I_2/g resin) actually loaded in polyanionic form on resin was determined by iodometric titration. To find effect of IO_3^-, anion exchange resin (Amberlite IR 400) was equilibrated with KIO_3 solution. Amount of IO_3^- loaded was estimated by iodometric titration[36] of initial solution and residual amount left after equilibration with resin. Only 0.061 g of IO_3^- was held per g of resin.

Test water samples (100 ml) were equilibrated with resin (4 g). Bacterial counts were estimated at initial time of addition (t=0) and then after keeping for 24 h. Samples were analyzed by standard MPN tests for TC and FC, H_2S strip test and plate count method. In plate count method, aliquots (1 ml) were taken out, serially diluted, and spread onto MacConkey agar plate and left in an incubator at 37°C for 24 h. Next day, plates were observed and bacterial colonies, if present, were counted using a colony counter and results expressed as number of colony forming units (CFU) per ml. For test on B-19 strain of *E. coli*, resin (4 g) was immersed into contaminated water (100 ml) with varying viable cell counts by different levels

Table 1—Evaluation of antimicrobial property of IO_3^- solution by MPN for FC and H_2S strip tests on STP sludge

S.No.	Treatment	Sample 1*		Sample 2*		Sample 3*		Sample 4*	
		MPN** (FC)	H_2S***	MPN (FC)	H_2S	MPN (FC)	H_2S	MPN (FC)	H_2S
1	Sterile H_2O (control)	0	'-'	0	'-'	0	'-'	0	'-'
2	Initial bacterial count in sample* (untreated water)	≥1100	'+'24 h	240	'+'24 h	93	'+'24 h	93	'+'24 h
3	1000 ppm IO_3^- in water sample	93	'-'	0	'-'	0	'-'	-	-
4	500 ppm IO_3^- in water sample	460	'-'	0	'-'	0	'-'	-	-
5	100 ppm IO_3^- in water sample	1100	'+'48 h	43	'+'48 h	0	'-'	3	'+'48 h
6	50 ppm IO_3^- in water sample	-	-	-	-	15	'+'48 h	15	'+'24 h
7	20 ppm IO_3^- in water sample	-	-	-	-	-	-	15	'+'24 h
8	10 ppm IO_3^- in water sample	-	-	-	-	23	'+'48 h	9	'+' 24 h
9	1 ppm IO_3^- in water sample	-	-	-	-	23	'+'48 h	23	'+'24 h

*Samples were drawn on different days and diluted to have bacterial count in measurable range; **MPN (FC) is most probable number for feacal coliform (FC). Results are given as MPN index/100 ml; ***Observation is qualitative indicate as '+'for black or '-'for no black colour

Table 2—Evaluation of antimicrobial property of NaCl (iodized commercial salt) solution by MPN for FC and H_2S strip test on STP sludge

S.No.	Treatment	Sample 2		Sample 3		Sample 4	
		MPN (FC)	H_2S	MPN (FC)	H_2S	MPN (FC)	H_2S
1	Sterile H_2O (control)	0	'-'	0	'-'	0	'-'
2	Initial bacterial count in sample (untreated water)	240	'+'24 h	93	'+'24 h	93	'+'24 h
3	1000 ppm NaCl (iodized commercial salt) in water samples	21	'+'24 h	0	'+'24 h	0	'+'24 h
4	500 ppm NaCl (iodized commercial salt) in water sample	21	'+'24 h	0	'+'24 h	0	'+'24 h
5	100 ppm NaCl (iodized commercial salt) in water sample	21	'+'24 h	7	'+'24 h	-	-
6	10 ppm NaCl (iodized commercial salt) in water sample	-	-	43	'+'24 h	-	-

of dilution. After different intervals of time, bacterial population was determined by plate count and MPN methods.

Results and Discussion

IO_3^- (conc., 1000 ppm) eliminated FC totally from samples 2, 3 and 4 (Table 1). However, in sample 1, which initially had ≥1100 bacteria, count was reduced to 93 bacteria in 24 h. In all cases, H_2S strip did not develop black colour, indicating effect of IO_3^- on sulphur reducing bacteria. Same trend was obtained at 500 ppm IO_3^-. Again, there was residual bacteria only in sample 1. At 100 ppm IO_3^-, trend was similar except that effectiveness was very low in sample 1 and increased in the order:

Table 3—Evaluation of antimicrobial property of NaCl (pure Merck) solution by MPN for FC and H_2S strip test on STP sludge

S.No.	Treatment	Sample 2		Sample 3		Sample 4	
		MPN (FC)	H_2S	MPN (FC)	H_2S	MPN (FC)	H_2S
1	Sterile H_2O (control)	0	'-'	0	'-'	0	'-'
2	Initial bacterial count in the sample (untreated water)	240	'+'24 h	93	'+'24 h	93	'+'24 h
3	5000 ppm NaCl (Merck) in water sample	240	'+'24 h	0	'+'24 h	0	'+'24 h
4	1000 ppm NaCl (Merck) in water sample	240	'+'24 h	7	'+'24 h	20	'+'24 h
5	500 ppm NaCl (Merck) in water sample	240	'+'24 h	15	'+'24 h	15	'+'24 h
6	100 ppm NaCl (Merck) in water sample	-	-	23	'+'24 h	-	-

Table 4—Evaluation of antimicrobial property of NaCl (Merck) and IO_3^- solution by MPN for FC and H_2S strip test on STP sludge

S.No.	Treatment	Sample 2		Sample 3		Sample 4	
		MPN (FC)	H_2S	MPN (FC)	H_2S	MPN (FC)	H_2S
1	Sterile H_2O (control)	0	'-'	0	'-'	0	'-'
2	Initial bacterial count in sample (untreated water)	240	'+'24 h	93	'+'24 h	93	'+'24 h
3	1000 ppm NaCl + 15 ppm IO_3^- in water sample	23	'+'24 h	4	'+'24 h	-	-
4	500 ppm NaCl + 100 ppm IO_3^- in water sample	-	-	6	'+'48 h	6	'+'48 h
5	500 ppm NaCl + 50 ppm IO_3^- in water sample	-	-	-	-	43	'+'24 h
6	500 ppm NaCl + 20 ppm IO_3^- in water sample	-	-	-	-	43	'+'24 h
7	500 ppm NaCl + 10 ppm IO_3^- in water sample	-	-	-	-	75	'+'24 h

sample 2 > sample 4 ≥ sample 3. Thus IO_3^- has shown antimicrobial properties but effect depends on initial level of bacterial contamination. For example, for sample 1, which had starting count ≥1100, up to 500 ppm, H_2S strip did not develop black colour and bacterial count came down to 460. At 100 ppm IO_3^-, both MPN and H_2S strip showed bacterial survival to a significant extent. On the other hand, in sample 2, where initial amount of bacteria was 240, IO_3^- was found effective up to 100 ppm with FC count coming down to 43. For an initial FC count to 93, as is the case of samples 3 and 4, IO_3^- was found effective up to 100 ppm.

Iodized salt (500 ppm) shows some antimicrobial properties when evaluated by MPN test (Table 2). On the other hand, H_2S strip indicated that, even at 1000 ppm, iodized NaCl has not inhibited sulphur reducing bacteria, may be because H_2S strip test responds to a consortia of sulphur reducing bacteria that may be present in sewage sludge. Probably FC from sewage is more susceptible to iodized salt than sulphur reducing bacteria.

Table 5—Evaluation of antimicrobial property of IO_3^- solution by MPN for TC on *E. coli* B-19 strain

S. No.	Treatment	Inoculum 1	Inoculum 2	Inoculum 3	
		MPN (TC)	MPN (TC)	MPN (TC)	Plate count1
	Sterile H_2O (control)	0	0	0	No colony
2	Initial bacterial count of sample (inoculum in water)	1100	1100	1100	Colonies present
3	1000 ppm IO_3^- in water sample	0	0	0	No colony
4	500 ppm IO_3^- in water sample	0	0	0	No colony
5	100 ppm IO_3^- in water sample	27	0	3	No colony
6	50 ppm IO_3^- in water sample	27	460	9	No colony
7	20 ppm IO_3^- in water sample	1100	≥ 1100	23	Colonies present
8	10 ppm IO_3^- in water sample	≥1100	1100	43	Colonies present

Table 6—Evaluation of antimicrobial property of NaCl and IO_3^- solution by MPN for TC on *E. coli* B-19 strain

S. No.	Treatment	Inoculum 1	Inoculum 2	Inoculum 3	
		MPN (TC)	MPN (TC)	MPN (TC)	Plate count method
1	Sterile H_2O (control)	0	0	0	No colony
2	Initial bacterial count of the sample (inoculum in water)	1100	1100	1100	Colonies present
3	500 ppmNaCl + 1000 ppm IO_3^- in water sample	-	0	0	No colony
4	500 ppm NaCl + 500 ppm IO_3^- in water sample	-	0	0	No colony
5	500 ppm NaCl + 100 ppm IO_3^- in water sample	27	23	3	No colony
6	500 ppm NaCl + 50 ppm IO_3^- in water sample	-	1100	240	Colonies present
7	500 ppm NaCl + 20 ppm IO_3^- in water sample	1100	≥1100	1100	Colonies present
8	500 ppm NaCl + 10 ppm IO_3^- in water sample	≥1100	≥1100	460	Colonies present
9	500 ppm NaCl + 1 ppm IO_3^- in water sample	≥1100	≥1100	≥1100	Colonies present

Looking into effect of pure NaCl (Merck) on bacterial consortia in water, even at 5000 ppm, there was no effect on sample 2, which had significant FC content initially (Table 3). In samples 3 and 4 with lower initial FC count of 93, salt (5000 ppm) showed antimicrobial property but no effect was seen on sulphur reducing bacteria. In all cases, H_2S strip became black.

In combination of NaCl and IO_3^- (Table 4), antimicrobial effect was seen for combination 3 (1000 ppm NaCl + 15 ppm IO_3^-) and 4 (500 ppm NaCl + 100 ppm IO_3^-).

Pure strain of *E. coli* B-19 gave negative test for FC and H_2S strip. So it was evaluated as TC by MPN method and plate count method (Table 5), using three inocula 1, 2, and 3 with initial bacterial count of 1100 index/100 ml by MPN. Results indicated that IO_3^- (100 ppm) inhibited growth. For salt and IO_3^- combination, inhibition could be seen up to 100 ppm of IO_3^- (Table 6).

Looking into antimicrobial effect of IO_3^- based resin on B-19 strain of *E. coli* in water (inoculum 1-4), bacterial counts do not differ significantly for resin loaded with IO_3^- and unloaded resin used as control (Table 7). Thus, IO_3^- when immobilized on resin did not exhibit significant antimicrobial properties. However, free IO_3^- exhibited antimicrobial properties (conc. > 100 ppm in water). Iodated resin evidently does not release this amount of iodate.

Test results on water procured from storm water drain flowing through IIT, Delhi, indicated that IO_3^- loaded resin was not effective (Table 8). However, with polyiodinated resin, bacteria (>10^6CFU/100ml) in highly contaminated water was completely killed in 24 h as MPN and plate count gave zero count, and H_2S strip did

Table 7—Effect of IO_3^- loaded Resin on *E. coli* B -19 strain

S. No.	Time h	Treatment	Inoculum 1 CFUx10^8/ml	Inoculum 2 CFUx10^7/ml	Inoculum 3 CFUx10^6/ml	Inoculum 4 CFUx10^4/ml
1	0	IO_3^- loaded resin(initial)	200	200	250	250
		Resin control (initial)	200	200	250	250
2	4	IO_3^- loaded resin	2	-	-	20
		Resin control	5	-	-	50
3	20 /24	IO_3^- loaded resin	0.6	4.5	0.74	2
		Resin control	0.6	86	0.8	4

Table 8—Effect of iodinated resin, IO_3^- loaded resin on drain water

S. No.	Treatment	Time	Type of tests		
		h	10^6 MPN index/100 ml	Plate count CFUx10^7/ml	H_2S strip
1	Iodinated resin	0	460	195	'+'24 h
		24	0	0	'-'
2	Iodated resin	0	460	195	'+'24 h
		24	460	188	'+'24 h
3	Resin control	0	460	195	'+'24 h
		24	460	200	'+'24 h

Table 9—Effect of iodinated resin on drain water

S. No.	Time	Treatment of drain water	Sample 1 Plate count CFUx10^4/ml	Sample 2 Plate count CFUx10^4/ml	Sample 3 Plate count CFUx10^4/ml
1	0 min	Drain water (initial count)	2	20	150
2	1 min	Iodinated Resin	-	No colony	No colony
		Resin control	-	9	80
3	5 min	Iodinated resin	No colony	No colony	No colony
		Resin control	-	8	40
4	10 min	Iodinated resin	No colony	No colony	No colony
		Resin control	-	6	50
		Drain water	-	9	-
5	1 h	Iodinated resin	No colony	No colony	No colony
		Resin control	-	7	30
		Drain water	0.7	6	100

not turn black. To find out minimum time required to kill all bacteria, iodinated resin (4 g) was shaken in a conical flask with drain water (100 ml) having an initial bacterial count (10^5-10^6 CFU/ml). Aliquots were drawn at different intervals and plating was done. Even within one minute, colony count came to zero in water in contact with polyiodinated resin whereas resin control gave a count of ~10^5CFU/ml (Table 9).

Iodinated resin was also tested for shelf life by repeatedly using sample in highly contaminated water. It continued to be effective even after a month with no loss of activity (Table 10); bacteria being killed within 1-10 min of contact.

Table 10: Effect of iodinated resin in repeated use on drain water

S. No.	Time	Treatment of drain water	1st use resin 100 ml drain water	Reused resin 100 ml drain water	Reused resin 100 ml drain water	Reused resin 100 ml drain water	Reused resin 100 ml drain water		`Reused resin 100 ml drain water		Reused resin 200 ml drain water	
			Plate count CFUx10^4/ml	Plate count CFUx10^4/ml	Plate count CFUx10^4/ml	Plate count CFUx10^4/ml	Plate count CFUx10^4/ml	H_2S strip	Plate count CFUx10^4/ml	H_2S strip	Plate count CFUx10^4/ml	H_2S strip
1	0 min	Drain water	150	4	4	4	5	'+'24 h	800	'+'24 h	10	'+'24 h
2	1 min	Iodinated resin	No colony	No colony	No colony	No colony	No colony	'-'	0.9	'+'24 h	0.03	'+'24 h
		Resin control	80	-	-	-	-	-	-	-	-	-
3	10 min	Iodinated resin	No colony	No colony	No colony	No colony	No colony	'-'	No colony	'-'	No colony	'-'
		Resin control	50	-	-	-	4	'+'24 h	95	'+'24 h	6.8	'+'24 h

Conclusions

Iodate (conc., >100-500 ppm in water) was effective in water disinfection, especially for less contaminated water. If this iodate level is present in common salt, it will help in disinfecting water during oral rehydration. Although commercial iodized salt contains less iodate, this could still disinfect water when initial bacterial contamination is low. IO_3^- loaded on an ion exchange resin did not exhibit antimicrobial properties probably because of low concentration of IO_3^-. On the other hand, polyiodide loaded resin was potent against indicator bacteria killing FC on contact and activity is retained over a long term.

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