‘Microorganisms in Re-circulatory Water Systems and Their Significance in Fouling’.

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ABSTRACT.

Microbiological pollution of water is of serious concern to mankind, not only because spread of diseases but also because of serious problems encountered due to microbially mediated corrosion and fouling, caused by biofilm formation. Biofilms are very common in natural and industrial water and if not controlled, may lead to fatal diseases as well as serious problems in the vital industrial operations. Legionella contamination of shower heads leading to respiratory illness are well documented along with the serious problems caused by iron and sulphur oxidisers and sulphate reducers in industrial corrosion and fouling.

Most of these microorganisms gain entry in water due to contamination from soil, sewage waste water, leaky pipelines discharges of animal dressings and carcasses, infected food stuff, pathological materials etc. Poor sanitation methods and haphazard usage of chemicals for the control of microorganisms has lead to significant resistance development. Recovery and repair of metabolic injury of microorganisms have, too contributed to the resistance.

Water and deposit samples collected from swimming pool and re-circulatory cooling systems from fertiliser, petrochemicals, refineries and pharmaceutical units were analysed for microbial contamination. These samples were highly contaminate, total viable bacteria counts were higher and enrichment studies showed presence of anaerobic bacteria, algae, S-oxidisers, Iron oxidisers, Sulphate reducers, Nitrifiers, Staphylococcus, Proteus species, coliforms, Pseudomonas, phenol and hydrocarbon degraders and fungi.

Efficiencies of biocides were determined to evaluate the microbial contamination control program using time kill and MIC tests. The biocide preparations containing Dichlorophene, Isothiozolone and MBT showed good reduction in microbial counts, however over a period these chemicals did not control the increase in counts as well arrest the growth of anaerobic bacteria. Peroxide complex prepared using silver and nonionic quat was very efficient in the control of microbial contamination. The resistance built up was absent in this complex as compared to other biocides tested.

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INTRODUCTION.

Microorganisms are found in water, air, soil as well as under extreme environmental conditions. They play vital role in rotation of elements in nature as well as maintenance of ecological balance. Adverse ecological changes are often reflected by increase in number of particular species over a period, Atlas (1980),Gardiner (1983),Lechavellier(1990)., Microbiological pollution of water is of serious concern to mankind, not only because spread of diseases but also because of serious problems encountered due to microbiologically mediated corrosion and fouling which are mainly caused by bio-film formation. Bio-films are very common in natural and industrial water and if not controlled, may lead to fatal diseases as well as serious problems in the vital industrial operations.

Cooling water systems represent a typical ecosystem which favours growth of microorganisms providing ideal environment and suitable nutrient balance for their multiplication. Natural associations like synergism, antagonism and predation are most prominent and favor survival and growth of organisms. Along with nutrient supply the re-circulation of water and different temp and pH zones are important for their survival and their multiple activities detrimental to the system and its functioning. Most of these microbes gain entry in cooling systems through air, water and soil.

Microbial fouling, together with corrosion and scaling in cooling water systems are the most common problems that damage expensive equipment, cause loss of production, increase in maintenance cost, reduce teat transfer efficiency and waste energy. Efficient and effective cooling water management lies in the dissipation of process heat at the most economical cost, while maintaining good heat transfer. Thus it becomes extremely important for one to study and understand all the dimension of cooling water chemistry, involving analysis, treatment, monitoring and control.

BIOFILM is an ideal environment for survival of bacteria. The bio-films are formed as results of gummy substances excreted by microorganisms, having a property of adherence. Over a period bio-film becomes thicker and thicker. Bio-film results into bio-fouling causing serious damages to surfaces, pipelines, water conducing systems in hospitals etc. Bio-films are reservoirs of pathogenic organisms giving protection against disinfectant action. The metabolic activities of these organisms results into formation of acidic environment which enhances the dissolution of material from the surfaces. The growth of microbes in the hairline cracks in the structures subsequently leads to the loosening of concrete material leading to the collapse of the same. Growth of algae enhances the discoloration and weakening of the concrete structures over a period, Hill (1984), Ridgway (1985),Angela (1996),Venkateswarlu (1997) VanderWende(1990).

Control of microorganisms in water is very important aspect of water management, so as to control not only the spread of epidemic disease but also microbiologically mediated corrosion causing heavy damages of the structures and equipment. Control measures involve proper monitoring measures for the detection of specific microorganisms responsible for the damages and use of chemicals to inactivate them. A suitable water audit program is very useful in deciding the water management system. The same must be decided depending upon the actual use of water in a particular application. Poor sanitation methods and haphazard usage of chemicals for the control of microorganisms has lead to significant resistance development. Recovery and repair of metabolic injury of microorganisms have, too contributed to the resistance, Hugo (1982), Grough (1982)

MATERIALS AND METHODS.

Studies were carried out in our laboratory to analyse water samples for microbiological quality. Water samples were collected from re-circulating cooling water systems from Petrochemical, Fertiliser and Pharmaceutical Industries and swimming pools.

Water samples collected in sterile containers were analysed for total viable bacteria counts, anaerobic bacteria, algae, S-oxidisers, Iron oxidisers, Sulphate reducers, Nitrifiers, Staphylococcus, Proteus species, coliforms, Pseudomonas and fungi. Depending upon the source of samples the microbial quality tests were conducted using methods as reported in Standard Methods for the examination of water and waste water (1992). Enrichment culture cultivation method using non selective enrichment followed by selective enrichment and isolation were adopted for the analysis. Results are reported in Table 1-3

Detailed analysis of deposit samples collected in sterile containers from the above industries were carried out using various microbiological methods as reported in Standard Methods (1992) results as shown in Table 4.

Biocides samples of peroxide-silver complex, Non ionic Quaternary ammonium compounds and its complex with peroxide,Dichlorophene, Isothializone and Methylene bis thiocyanate (MBT) based were used for evaluation using time kill and minimum inhibitory tests in conventional culture media, McCoy (1980). The peroxide silver complex was prepared by mixing silver concentrate with 50% Hydrogen peroxide so as to give final level of silver in the complex about 500ppm. The peroxide quat complex was prepared by mixing non ionic quat with 50% Hydrogen peroxide so as to give final level of quat in the complex about 0.5%. the preparation were carried out in glass reactor with stirring arrangements.

The studies were carried out in artificially prepared cooling water typically representing each selected industries, contaminated with microorganism isolated during the studies. Each selected biocide was added at concentrations as shown in tables to the set of contaminated water sample prepared by adding the enriched bacteria, the TBC/ml were determined at different interval. In case of algae and anaerobic sulphate reducing bacteria the evaluation were based on delay in the period of growth over a incubation period.Results are as shown in Table 5-6.

Silver Peroxide complex was evaluated for its efficacy to kill intestinal organisms like E.coli and V.cholerae,results vide Table-7.

Complexed peroxide with silver and non ionic quat were also evaluated for its efficacy in treatment of swimming pool water samples prepared in laboratory, results vide Table-8.

RESULTS.

The water samples and deposit samples collected from re-circulatory cooling systems from Fertiliser, Petrochemicals Refineries and Pharmaceutical Industries were found to be contaminated with wide range of bacteria and many of them showed the presence of Pseudomonas, Desulfovibrio, Nitrifiers, Phenol and hydrocarbon degraders, Iron oxidisers and variety of blue green bacteria, green algae and fungi belonging to Deuteromycetes and Ascomycetes group. The number of anaerobic Desulfovibrio was more in sump water then in return line. However deposit samples had sessile forms of this bacteria. The Desulfovibrio when cultivated in medium with shoe nails immediately showed heavy growth on the same indicating their sessile nature. Vide tables 1-4

The corrosive effects of these organisms were clearly visible on the tower packing as well as the heat exchangers surfaces were pitted with reduction in the thickness of the metals. The TBC/ml were $>10^7$,however immediately after addition of the biocides the same were reduced. The inactivation of the biocides were significant as observed by increase in TBC/ml after 24hrs of dosing.

The efficacy of biocides were tested using MIC, % kill of bacteria and delay in growth of bacteria.tables 5-6 shows the effect of various biocides at different concentration on bacterial counts after 24,48,and 72hrs of contact time. From these tables it can be seen that immediately after dosing biocide the reduction in TBC were observed with each biocide, however over the incubation period the number of bacteria increased in case of Dichlophene, MBT and Isothiazole , while in case of peroxide complex the increase was much less as compared to others. The survival of Desulfovibrio and algae in case of peroxide and non ionic quats was much less and both these delayed the growth over 20 day. This is significant as in any cooling water treatment the biocide dosages are normally at a interval of 2-5 days.

From the Table-7 it can be seen that the peroxide silver complex is very effective in killing E.coli and V.cholerae and in a contact time of <60mins the reduction in viable counts were 100% even at 30ppm.

Table-8 shows the results as observed for swimming pool water treatment using peroxide complex with silver and non ionic quat respectively.. It can be seen from the table that both these formulation were found to be very effective in reducing bacterial counts >99% in a contact time of 60mins and the counts were under control for 48hrs. At a concentration of 35ppm of each complex the growth of coliforms, E.coli and other pathogen was arrested.

Studies on the biocide applications revealed the development of resistance in bacteria towards the quaternary ammonium compounds and phenolic compounds. The efficacy of chlorination to control bio-films was very poor. The efficacy of peroxide silver preparation and peroxide and non ionic quaternary ammonium compounds were extremely good in killing both free living and sessile bacteria.

Table -1.
Bacteriological Analysis of Cooling Water Samples.

Microbiological Tests	Samples collected from Fertiliser Units		
	Urea Tower	Ammonia Tower	Steam generation
Total Bacteria counts/ml	$5x10^6$ - 10^8	$3x10^6$ - 10^7	$6x10^6$ - 10^7
SRB/100ml by MPN	2400	1100	1100
Algae(Blue green Bacteria)	Present	Present	Present
Iron Oxidisers	Present	Present	Present
Iron Bacteria	Present	Present	Present
Nitrifiers/100ml by MPN	>2400	>2400	>2400

Table -2.
Bacteriological Analysis of Cooling Water Samples.

Microbiological Tests	Samples collected from		
	Refinery	Pharmaceutical	Petrochemical
Total Bacteria counts/ml	$5x10^{6}$ - 10^{8}	$3x10^{6}$ - 10^{7}	$6x10^{6}$ - 10^{7}
SRB/100ml by MPN	2400	1100	1100
Algae(Blue green Bacteria)	Present	Present	Present
Iron Oxidisers	Present	Present	Present
Iron Bacteria	Present	Present	Present
Phenol degraders/ml	400-500	200-300	1500-2000

Table -3.
Bacteriological Analysis of Swimming Pool Water Samples.

Microbiological Tests	Samples collected from		
	I	II	II (salt water)
Total Bacteria counts/ml	100-150	150-250	400-500
Coliforms/100ml by MPN	4	7	11
Pseudomonas spp.	Absent	Absent	Absent
Staphylococcus spp.	Absent	Absent	Absent
Proteus spp.	Absent	Absent	Absent
Salmonella spp.	Absent	Absent	Absent

Table-4A

Bacteriological Analysis of Deposits collected from Fertiliser Unit.

UREA TOWER	AMMONIA TOWER	BLOW DOWN DISCHARGE
Nitrobacter,Nitrosomonas	Pseudomonas	Pseudomonas
Ammonifiers	Nitrobacter and Nitrosomonas	Desulfovibrio
Denitrifiers	Bacillus.	Coliforms.
Filamentous bacteria	Coliforms.	Bacillus.
Pseudomonas	Iron oxidisers	Iron bacteria
Fe Bacteria	Blue green bacteria	Bluegreen bacteria
Desulfovibrio spp.	Desulfovibrio	Filamentous.
Clostridium spp.	Clostridium spp.	Clostridium spp.

Table – 4B.

Bacteriological Analysis of Deposits collected from Fertiliser Unit.

PHARMACEUTICAL	PETROCHEMICAL	REFINERIES	SWIMMING POOL
Nitrifiers	Pseudomonas	Pseudomonas spp.	Staphylococcus spp.
Ammonifiers	Desulfovibrio spp.	Desulfovibrio spp.	Escherichia spp.
Denitrifiers	Oil degraders	Oil Degraders.	Bacillus spp.
Filamentous bacteria	Phenol degraders	Phenol Degraders	Blue green bacteria.
Pseudomonas	Iron oxidisers	Iron oxidisers.	Filamentous bacteria
Fe Bacteria	Blue green bacteria	Fe bacteria	
Iron precipitators.		Filamentous Bacteria.	

Table-5.

Effect of various Biocides Total Bacteria counts in water samples.

Biocide	Concentration in ppm	0hrs.	24hrs.	48hrs.	72hrs.
Non ionic quat	5 10	$2.8x10^7$ $2.8x10^7$	$2.0x10^4$ $2.0x10^3$	$4.0x10^4$ $5.2x10^3$	$2.0x10^5$ $6.0x10^4$
Peroxide silver	30 60	$3.0x10^7$ $2.9x10^7$	$1.6x10^4$ $2.0x10^3$	$3.0x10^4$ $4.2x10^3$	$2.0x10^5$ $1.8x10^4$
Dichlorophene	50 100	$2.6x10^7$ $2.8x10^7$	$2.2x10^5$ $1.8x10^4$	$6.8x10^5$ $5.0x10^4$	$4.8x10^6$ $8.0x10^5$
Isothiozolone	50 100	$2.8x10^7$ $2.7x10^7$	$1.6x10^5$ $2.4x10^4$	$7.2x10^5$ $8.0x10^4$	$2.4x10^6$ $5.0x10^5$
MBT based	50 100	$2.5x10^7$ $2.6x10^7$	$2.2x10^5$ $2.4x10^4$	$6.8x15^5$ $7.2x10^4$	$2.4x10^6$ $6.2x10^5$

Table-6

Effect of Biocides on growth of SRB and Algae.

Biocide	Concentration in ppm	Delay in growth of SRB in days.	Delay in growth of Algae in days.
Non ionic Quat	5 10	10 16	13 22
Peroxide silver Complex	30 60	12 18	16 21
Dichlophene	50 100	7 12	10 17
Isothialozone	50 100	10 14	14 20
MBT based .	50 100	14 20	12 18

Table-7

Effect of Peroxide Silver complex on Intestinal pathogens.

Conc. of complex (ppm)	Time of exposure (minutes)	*E. Coli*		*V. cholerae*	
		count/ml	% kill	Count/ml	% kill
NIL (control)	0	1200	0.00	2100	0.00
25	30 60 120	46 29 NIL	96.17 97.58 100.00	NIL NIL NIL	100.00 100.00 100.00
50	30 60 120	46 26 NIL	96.17 97.83 100.00	NIL NIL NIL	100.00 100.00 100.00
70	30 60 120	32 12 NIL	97.33 100.00	NIL NIL NIL	100.00 100.00 100.00

Table - 8

Effect of Peroxide Complex on Survival of Bacteria in Swimming pool Water.

TEST.	Contact Time in Mins.	Peroxide and Silver Complex.		Peroxide and non ionic Quat complex	
		30ppm	60ppm.	30ppm	60ppm.
TBC/ml	0. 30. 60. 24hrs	1500-1800 250 Nil. Nil.	1500-1800 60 Nil Nil	1500-1800 210 Nil Nil	1500-1800 55 Nil Nil
Coliforms MPN/100ml	0. 30. 60. 24hrs	240 Nil Nil Nil	240 Nil Nil Nil	240 Nil Nil Nil	240 Nil Nil Nil

DISCUSSION.

Today's high technology water purification systems are designed to remove many types of impurities. One of the most difficult to deal with is microbiological contamination. Microbial growth in a water systems presents two problems: it not only reduces water quality, but also if left unattended, the problem can 'grow' to reduce system performance and life. Bio-fouling can cause a decrease in product water flow rate. a decrease in driving pressure, and can contribute to corrosion of piping and housing, Paul (1997), USEPA (1993),Chambers (1962).

The commonly observed problem of cooling water systems are mainly due to atmospheric environment and water quality. The physical and chemical nature of water system play a key role in both functioning and corrosion of the system. Corrosion and scaling due to chemical quality of water used are mainly controlled by using various chemicals. However many of these chemicals serve as very good food for microorganisms and allow them to grow and multiply in the system. Thus microbial fouling and corrosion normally occurs in-spite of proper chemical treatment.

Most of these problems are due to variety of nuisance bacteria entering in the system through various sources. The most commonly encountered bacteria in re-circulatory water systems are species of Bacillus,Proteus, Pseudomonas,Escherichia,Enterobacter,Salmonella,Staphylococcus,Thiobacillus,Flavobacterium,Sphaerotillus, Ga-llionella,Leptothrix,Ferrobacillus,Nocardia,Streptomyces,Nitrobacter,Nitrosomonas,Azotobacter,Legionella, Desulfovibrio,Clostridium etc.

Among fungi are species of Asperigillus,Penicillium,Chaectomium,Cladosporium, Basidiomycetes etc, while among algae are species of Anabena, Nostoc, Oscillitoria, Ulothrix, Chlorella and many more.

The analysis of water and deposit samples showed the presence of many such organisms. They were found to be responsible for pitting and scaling of metal surfaces and fouling of water systems as evident from discoloration and foul smell in-spite of the chemical quality of water used in re-circulation being maintained within normal limits. In case of swimming pool water the slimy deposits from the tiled surfaces revealed the presence of E.coli,Proteus and Staphylococcus by enrichment method although the TBC were in normal range.

Anaerobic sulphate reducing bacteria like Desulfovibrio play a very important role in the fouling leading to the graphitization and perforation of iron pipelines, scaling and pitting on the surfaces of heat exchangers due to H_2S production. Activity of SRB leads to malodorous black slime indicating profuse bacterial activity. The deposit sample revealed the presence of highly active SRBs and Clostridium species.

Bacterial population in re-circulatory water systems can exceed one million per ml and the best levels that should be achieved in conventional treatment program are in thousands. According to the Center for Disease Control, when bacterial populations reach or exceed 500,000 colonies per ml there is an enhanced risk of Legionella growth. High levels of bacteria can also pose increased risks for microbiologically influenced corrosion and the formation of bio-film on wetted surfaces. It has been also observed that the bio-film can protect the inner layers from the biocides being applied. Under this situation the lower layers become deficient in oxygen allowing heavy growth of anaerobic bacteria which notably includes SRBs and iron metabolizing bacteria. The sulphate – reducing bacteria produce acid from their reduction of sulphate,iron metabolizing bacteria can literally consume iron to provide for metabolic needs and thus both these bacteria can destroy Schedule 80 iron piping in as little as nine months,McCoy(1980),Postgate (1979)Ozone Treatment (1995),Bull (1991).

The total viable bacteria counts were > 105/ml after 24hrs of biocide dosing, the bacterial counts increased after 2-3 days indicating inhibitory action of biocides used were temporary while the killing of anaerobic bacteria was very poor. The fungal and algae growths were temporarily arrested.

Microbial fouling with direct or indirect corrosive effects and interference in re-circulation is controlled by using chemicals collectively known as biocides, which have either cidal of inhibitory action on microorganisms. Various factors influence biocidal activities of these chemicals and among these development of resistance and immunity is the most important one and is mainly responsible for inactivation of biocides. Bacteria can develop resistance to destroy biocides by (i)producing enzymes (ii)change in internal structure of cell (ii) change in permeability of the cytoplasmic memebrane and (iv) change in the composition of cell wall and mutations and genetic transfer.

In general biocides are selected after proper efficacy trials which are carried out in laboratory cooling tower or flasks.The efficiency of various biocides under tests were evaluated by determining their MIC and % kill of bacteria counts as well as their ability to delay the growth of algae and SRBs. Immediately after 50 and 100ppm respectively dosage of selected biocide for the trials the bacterial counts reduced <99% in 24hrs of contact time, however in case of Dichlophene, Isotialazone and MBT based biocides the bacterial counts showed increasing trend over a increasing contact time. The peroxide complexed with silver and non ionic quats showed excellent results. Both these products at 50 and 100ppm dosage effectively arrested the growth of Algae and SRB indicating their usefulness in controlling corrosion and fouling. The degree of resistance development towards selected biocide when tested revealed that in case of Dichlorophene, Isothialazone and MBT the MIC of these biocides changed and showed increase in inhibitory concentration with respect to aerobic and abnaerobic bacteria.

The complex tested for swimming pool at 30 and 60ppm dosage gave excellent results as compared to conventional chlorination. The water samples when examined by enrichment methods did not show presence of any coliforms and pathogens. The bacterial counts were maintained within limits and the increase in the counts were insignificant.

CONCLUSION

- The microbial quality monitoring program as studied in different water samples based on their end use clearly showed presence of wide variety of microbial contamination causing serious problems in the system.

- The dangers of health hazards may be significant if one restricts the analysis of water sample to the routine contaminants.

- The studies also indicated that the monitoring program should include the analysis of samples for various intestinal pathogens, Pseudomonas Legionella, Desulfovibrio and many others along with the routine types.

- The swimming pool water samples may be analysed for various pathogens like Proteus,Staphylococcus,Salmonella, Enterobacter, Desulfovibrio and Clostridium. Periodic analysis of slimy material from pool reservoir and filter assembly must be carried out to reveal the presence of sessile forms.

- The biocide dosing program should include the effect of the desired chemical on both free living and sessile forms and to avoid any resistance being built up the rotation of biocide should be recommended.

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