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Studies on the Degradation of Calcium from Water using Microorganism

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Cooling water system plays a vital role in thermal power plants. Cooling water system removes the heat via evaporative coolers. Its primary aim is to remove the heat absorbed in the circulating water system. Water is pumped from the tower base into cooling water routed through the process cooler and condenser. The cool water absorbs the heat from hot process system. The absorbed heat warms the circulating water, which returns to the top of cooling tower and tickles downward towards the materials inside the tower. The evaporated water leaves the dissolved salts in the water which has not been evaporated thus salt concentration is increased in water. The concentration of dissolved salts in the cooling water exceeds their solubility limits and precipitates on the water surface and forms the scale. The most common scale formers, calcium salts cause scale formation in the most sensitive areas of heat transfer. In extreme cases, enough material precipitates physically blocked the cooling water passages, resulting in the affected equipment being removed from the operation for either chemical or mechanical cleaning.. The microbes, which are ubiquitous in nature, particularly the bacteria utilises some of the substrates as sole source for their multiplication; sometime it may utilize a special component and can degrade the other substances.. Thus the production of enzyme urease by some bacteria plays a vital role in reducing calcium by utilizing the anural substrate urea. The primary role of bacteria in the precipitation process has been ascribed to their ability to create an alkaline environment through various physiological activities. Immobilization of the microbes (biocatalysts) in specific matrices will further improve the efficiency of the process.

Key words: Cooling tower, scales, calcium, immobilization, *Bacillus cereus, Pseudomonas fluorescence, Staphylococcus aureus*

Water is a common chemical substance that is essential for the survival of all known forms of life. In typical usage, water refers only to its liquid form or state, but the substance also has a solid state, ice, and a gaseous state, water vapour or steam. Water covers 71% of the Earth's surface .Water is a very strong solvent, referred to as "The Universal Solvent" dissolving many types of substances. Substances that will mix well and dissolve in water, e.g. salts, sugars, acids, alkalis, and some gases: especially oxygen, carbon dioxide (carbonation), are known as "hydrophilic" all the major components in cells (proteins, DNA and polysaccharides) are also dissolved in water (Kotz *et al.*, 2005).

Mercury - 3.4% in the atmosphere, and large amounts of water in Mercury's exosphere (Astonished, 2003). Venus - 0.002% in the atmosphere. Earth - trace in the atmosphere (varies with climate). Mars - 0.03% in the atmosphere. Jupiter - 0.0004% in the atmosphere. Saturn - in ices only. Escalades (moon of Saturn) - 91% in the

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atmosphere. Explants known as HD 189733 b (Laura blue, 2007). Earth's approximate water volume (the total water supply of the world) is 1 360 000 000 km^3 (326 000 000 mi^3) (Dooge and Ehlers, 2001). Water and steam are used as heat transfer fluids in diverse heat exchange systems, due to its availability and high heat capacity, both as a coolant and for heating. Condensing steam is a particularly efficient heating fluid because of the large heat of vaporization. In almost all electric power plants, water is the coolant, which vaporizes and drives steam turbines to drive generators. Water is used in power generation. Hydroelectricity is electricity obtained from hydropower. Hydroelectric power comes from water driving a water turbine connected to a generator. Hydroelectricity is a low-cost, non-polluting, renewable energy source. Water is also used in many industrial processes and machines, such as the steam turbine and heat exchanger (Ravindranath, *et al.*, 2002).

Aim & objectives

1) To isolate and identify the calcium degraders from calcium rich source.
2) To estimate the amount of calcium in the makeup water of thermal power station
3) To analyze for some other water parameters such as pH, conductivity, solids, sulphates, Iron, cations and Anions (volumetric methods and gravimetric methods).
4) To determine the rate of degradation of calcium in water sample through microbes.

The present study was mainly focused on the removal of calcium using microbes, ions like calcium which is accumulated a large amount is capable of causing scaling along pipeline, which tend to reduce the power production. For the reduction of calcium, number of methods is employed, which are mostly of chemical method. The chemical method is one of the classical method which removes calcium effectively. In the present scenario chemicals such as organophosphate mimics with the calcium under suspension of water, it is also expensive. To overcome these problems, an efficient method was used as microbes to predict the calcium removal from the cooling water using biological approach. From these bacteria (*Bacillus cereus, Staphylococcus aureus and Pseudomonas fluorescence*) seem to be novel products and its shows 100% efficiency evaluated by continuous, batch and immobilization methods.

Materials and methods

Sample for isolation of bacteria

The bacteria which are capable of reducing calcium were isolated from calcium rich areas like Mine field, Sea shore from the samples like - Limestone, Mine spoiled soil, Sea shell

Sample used for water treatment:

The sample to be treated with calcium reducing bacteria to reduce the calcium content was collected from thermal power station. Make up water of thermal power station

Pure culture technique

To study the characteristics of single species, that particular species must be separated from all other species, through pure culture. (Hence pure culture is needed) Pure culture is a population of uses arising from a single cell to characterize an individual species.

Pure culture techniques involves the followings

Serial dilution, Spread plate technique, Streak plate technique

Method of serial dilution

The sample [mine spoiled soil, sea shell powder] was suspended in sterile distilled water aseptically. From this, 1ml of sample was pipette out into a test tube containing 9ml of sterile distilled water to obtain 7 dilutions were made up to 7. 0.1ml of appropriate dilution was transferred into sterile petriplate for spread plate technique.

Spread plate technique

Nutrient agar with urea medium was prepared and sterilized and poured into the sterilized petriplate and allowed to solidify. The given sample was serially diluted from 7 to 7. From each dilution 0.1ml of sample was transferred into sterile petriplate with the help of the sterile micro pipette. The 0.1ml of the sample present over the surface of the medium was spreaded evenly by using L-rod; plates were incubated at 37°C for 24 hours to get isolated colonies.

Streak plate technique

Pure colonies also are used in streak plate techniques. The microbial mixture was transferred in to the edge of the agar plate with an inoculating loop and then streaked out over the surface in the following patterns of Quadrants streaking. This inoculation "Thin out" the bacteria and they are separated from each other. The plates were incubated at 37°C for 24 hours.

RESULT AND DISCUSSION

The makeup water of power station contain all the minerals along with calcium, but the increase in calcium is considered to be reducing the quality of pipe line, by causing scaling. So it is important to reduce the calcium level. The present study was carried out with biological removal process as well as chemical method. The chemical method has certain disadvantages, such as highly expensive. The sea shell, mine spoiled soil, lime stone, the calcium rich sources contains several bacteria which are capable of reducing the calcium, by ureolytic microbial calcium carbonate precipitation.

Table 1. Calcium reduction by continuous method using *Pseudomonas fluorescence*

S No	Urea concentration%	Initial calcium (ppm)	Calcium available after 2hrs (ppm)	Calcium available after 4hrs (ppm)	Calcium available after 6hrs (ppm)
1.	0	300	298	295	293
2.	1	300	297	292	288
3.	2	300	296	291	286
4.	3	300	290	286	284
5.	4	300	285	280	276

Table 2. Calcium reductions by continuous method using *Staphylococcus aureus*

S No	Urea concentration%	Initial calcium (ppm)	Calcium available after 2hrs (ppm)	Calcium available after 4hrs (ppm)	Calcium available after 6hrs (ppm)
1.	0	300	299	296	290
2.	1	300	297	293	289
3.	2	300	296	290	286
4.	3	300	294	287	282
5.	4	300	291	285	279

Table 3. Calcium reductions by continuous method using *Bacillus cereus*

S No	Urea concentration%	Initial calcium (ppm)	Calcium available after 2hrs (ppm)	Calcium available after 4hrs (ppm)	Calcium available after 6hrs (ppm)
1.	0	300	299	295	293
2.	1	300	297	281	278
3.	2	300	295	276	257
4.	3	300	295	274	254
5.	4	300	294	269	251

Table 4(a). Water treatment by immobilization technique Sample : Ground water

S. No.	Organisms	Initial amount of calcium (ppm)	After 2 hours		After 4 hours		After 6 hours	
			Available calcium (ppm)	Reduced %	Available calcium (ppm)	Reduced %	Available calcium (ppm)	Reduced %
1.	*Pseudomonas fluoresecence*	44.8	42.1	6	36.2	19	31.8	29
2.	*Staphylococcus aureus*	44.8	42.1	6	33.6	25	30.1	33
3.	*Bacillus cereus*	44.8	40.7	9	24.5	45	8.9	80.1

Table 4(b). Water treatment by immobilization technique Sample : Sump water

S. No.	Organisms	Initial amount of calcium (ppm)	After 2 hours		After 4 hours		After 6 hours	
			Available calcium (ppm)	Reduced %	Available calcium (ppm)	Reduced %	Available calcium (ppm)	Reduced %
1.	*Pseudomonas fluoresecence*	88	82.7	7	71.2	19	61.6	30
2.	*Staphylococcus aureus*	88	81.8	6	67.7	23	59.8	32
3.	*Bacillus* cereus	88	80.5	8.5	51.0	42	21.2	76

Table 4(c). Water treatment by immobilization technique Sample: Blow down water

S. No.	Organisms	Initial amount of calcium (ppm)	After 2 hours		After 4 hours		After 6 hours	
			Available calcium (ppm)	Reduced %	Available calcium (ppm)	Reduced %	Available calcium (ppm)	Reduced %
1.	*Pseudomonas fluoresecence*	256	238.1	7	204.8	20	197.1	23
2.	*Staphylococcus aureus*	256	236.8	7.5	195.5	23	181.1	29

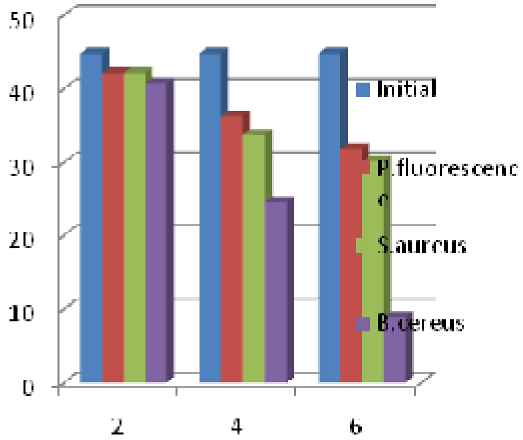


Fig 1. Calcium reduction in ground water by immobilization technique

Calcium reduction by continuous method using the isolates:

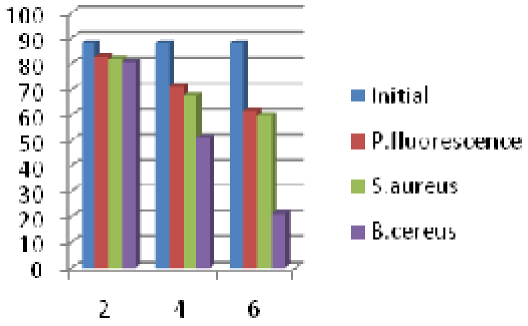
-Bacillus cereus, Pseudomonas fluorescence, Staphylococcus aureus.

The ability of the organisms isolated were tested through continuous method, where in a known amount of calcium (300 ppm as calcium chloride) was added to water and the bacterial culture was inoculated into it. The growth of the organisms was increased by continuous agitation by shaking. The rate of calcium reduction was measured at regular interval (2hrs, 4hrs and 6hrs). The efficiency was more with *Bacillus* at 6hrs. [Shown in *Table 1, 2&3 Figure-1*]

Water treatment by immobilization technique Samples

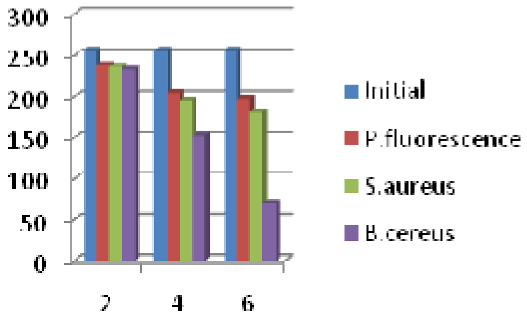
Ground water, Sump water, Blow down water

In the immobilization method the individual organisms were entrapped with in calcium beads, the reduction rate was measures by filling the beads in column reactor. The beads were found to be stable at 4% sodium alginate concentration. The 20 ml of 2% urea was allowed to react with the entrapped organisms, which shows effective for *Bacillus*. The rate of the water through the column reactor with beads was 3ml per minute. The water was recycled continuously. This was followed for each sample waters and for each organism separately. The sample was collected at every 2hrs up to six hours and tested for calcium reduction in it. *Bacillus cereus* organism was found to reduce the calcium better than the other two in all the sample water. Hence the immobilization method with *Bacillus cereus* organism is preferable for any other method of calcium reduction in water through biological means. [Shown in *Table 4(a, b&c) Figure-1*]



X-axis- time in hours Y-axis- calcium concentration in ppm

Fig. 2. Calcium reduction in sump water by immobilization technique



X-axis - time in hours Y-axis - calcium concentration in ppm

Fig. 3. Calcium reduction in blow-down water by immobilization technique

CONCLUTION

The present study revealed that the efficiency of biological removal of calcium might be increased by selection of organisms. The organism also is changed, through mutation. The organism should be selected, such that it should posses the following characters. Such as it should not alter P^H, reduction should be high and incubation period should be low. Economically it is more beneficial and should not alter any other water quality. Makeup water for used cooling the evaporative condensers contain dissolved solids, organic compounds and suspended solids. These problems were caused in cooling water systems as they increase in concentration as the water evaporates in the cooling process. The most common problems are scaling and fouling. Scaling was formed by the accumulation of calcium in the sides of the water treatment equipment and interferes with the water flow and productivity of cooling tower. To overcome these interferences, the calcium present in the cooling water should be removed or reduced. Chemical and biological methods are suggested to reduce calcium present in the makeup water. Among these two methods, biological method is considered as a convenient method due to the reason of certain demerits in chemical method such as highly expensive, time consuming and water pollution. Ureolytic calcium precipitating microorganisms are recommended for calcium reduction.

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