AMET International Journal of Physical & Chemical Sciences, AJPCS Vol. **1**(issue 1), p. 23-35

STUDIES ON THE DEVELOPMENT OF BIOCATALYST FOR REMOVAL OF CALCIUM FROM WATER

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(Received: 15 August 2014; accepted: January2014)

ABSTRACT

One of the major problems that occur in a thermal power station is the formation of scales inside the cooling towers. 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 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. Number of bacteria produces various bioproducts, enzymes; which are responsible for the cleavage of complex compound into simpler ones. Thus the production of enzyme urease by some bacteria plays an 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, antiscalants, microbes (bacteria), urease/urea, immobilization.

INTRODUCTION:

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 vapor or steam*. Water covers 71% of the *Earth*'s surface (Kulshreshtha, 1998)[1]. *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. *Enceladus* (moon of Saturn) - 91% in the atmosphere. exoplanets 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 km³ (326 000 000 mi³) (Dooge and Ehlers, 2001)[2].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)[3]. Ground water is generally less susceptible to contamination rather than surface waters. Ground water is usually more highly mineralized in its natural state. Water physical parameters, such as electrical conductivity, P^H and major iron concentrations such as Ca, Mg, Na, Ka, Cl, HCO_3, CO_3 and SO_4 of ground water were taken into consideration. Concentration of these cations and anions in the ground water vary spatially and temporally. Abundance of these ions is in the following order. Ca> Na> Mg> K= HCO_3 > C1 > CO_3 > SO_4. Ca – HCO_3 and Ca – C1 – HCO_3 are the dominant hydro chemical fancies of the study environment. Cations such as calcium, magnesium, sodium and potassium, ammonia and anions such as chloride, bicarbonate, sulfate, bromide, fluoride, and nitrate are active components present in the industrial water (Lakshamanan *et al.*, 2003)[4].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. (Demadis and Kostas ,2004)[5].

AIM & OBJECTIVES:

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

MATERIALS AND METHODS:

SAMPLE COLLECTION

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 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 spread evenly by using L-rod; plates were incubated at 37°C for 24 hours to get isolated colonies.

STREAK PLATE TECHNIQUE:

Pure colonies also 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.The above samples were inoculated into the nutrient agar plate containing urea. The bacteria produce clear zone around their colonies along the medium. From this three different types of bacteria were isolated (isolate1, 2 and3).[*shown in Figure-2*]

GRAM STAINING:

The gram staining which is the primary microscopic technique was done and were observed under microscope [shown in *Table-1*,*Figure-3*]

MOTILITY TEST:

The motility test was carried out for the observation of motility among the isolated organisms, both motile and non-motile organisms was observed. [Shown in *Table-1*]

CATALASE TEST:

Evaluation of air bubbles indicates the production of catalyses enzyme [shown in *Table-1*]

OXIDASE TEST:

Determination of the isolated *bacterium* to produces certain *cytochrome c oxidases* was confirmed by oxidase test, development of blue colour indicates oxidase production which is positive,for negative results no blue colour formation was observed [shown in *Table-1*,]

INDOLE TEST:

Indole test done for the tryptophan aminoacid utilization test showed a disappearence of cherry red colour [shown in *Table-1,Figure-5*]

METHYL RED TEST:

Appearance and disappearence of pink colour was observed in a test for the glucose utilization called methyl red test [shown in *Table 1,Figure-5*]

VOGES PROSKAUER TEST:

The production and presence of non acidic and neutral end products as a result of glucose metabolism are tested by voges proskauer method. [shown in *Table 1,Figure-5*]

CITRATE UTILIZATION TEST:

Production of citrate permease enzyme was identified by citrate utilization which resulted in the appearance and disappearance of green to deep Prussian blue [shown in *Table 1,Figur*e-5]

UREASE TEST:

After incubation, a pink to red color constitutes a positive test. If the original straw color persists, the test is negative. [shown in *Table 1,Figure-5*] The three isolates were confirmed as *Bacillus cereus, Pseudomonas fluorescence, Staphylococcus aureus* respectively

TABLE:1 IDENTIFICATION AND CHARACTERIZATION OF MICRO ORGANISMS

Tests	***Bacillus cereus***	***Pseudomonas fluorescence***	***Staphylococcus aureus***
Gram staining	Positive rod	Negative rod	Positive cocci
Motility test	Positive	Positive	Negative

Catalase test	Positive	Positive	Positive

Serial no	**Test Conducted**	**Ground Water (ppm)**	**Sump Water (ppm)**	**Blowdown water (ppm)**

Oxidase test	Positive	Negative	Negative
Indole test	Negative	Negative	Negative
Methyl red test	Negative	Negative	Positive
VogesProskauer test	Positive	Positive	Negative
Citrate test	Positive	Positive	Negative
Urease test	Positive	Positive	Positive

TABLE:2 WATER ANALYSIS

1.	Calcium as Ca	44.8	88	256
2.	Magnesium as Mg	22.3	35.96	122.3
3.	Sodium as Na	107.14	103.50	678
4.	Iron as fe	0.76	0.6	1.5
5.	Silica as SiO_2	38.4	102.40	165
6.	Chloride as Cl	62	98	713
7.	Sulphate as So_4	160.1	174.3	233
8.	Total solids	393	398	2550
9.	Dissolved solids	355	358	2500
10.	Suspended solids	38	40	50
11.	Total alkalinity	76	236	350
12.	Bicarbonate alkalinity	76	23	25
13.	Total hardness as $CaCo_3$	204	368	351
14.	Conductivity in microbes	549	1092	3350
	temperature	6.64	7.43	7

TABLE:3 Calcium reduction by continuous method using *Pseudomonas*

Serial 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:4 Calcium reduction by continuous method using *Staphylococcus aureus*

Serial 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

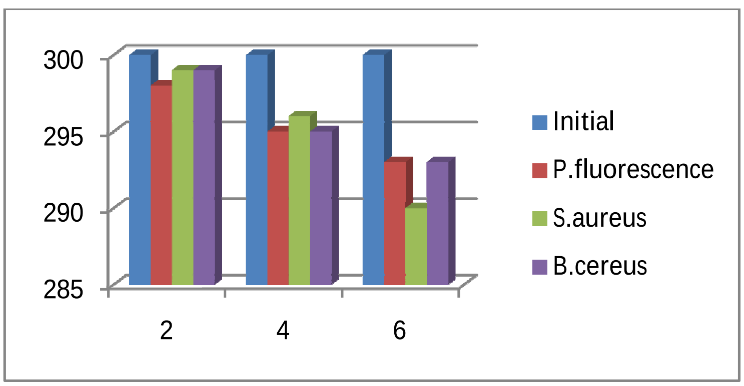
TABLE:5 Calcium reduction by continuous method using *Bacillus cereus*

Serial 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

CALCIUM REDUCTION BY CONTINUOUS METHOD

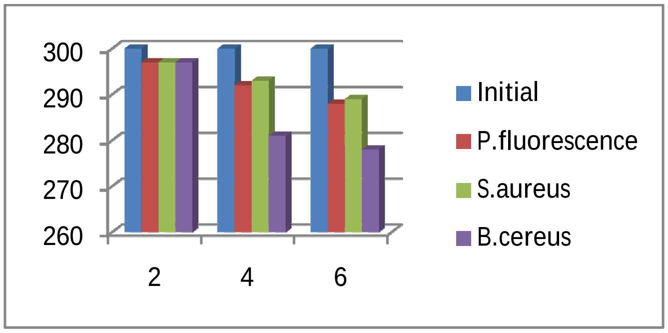
At 0% urea concentration



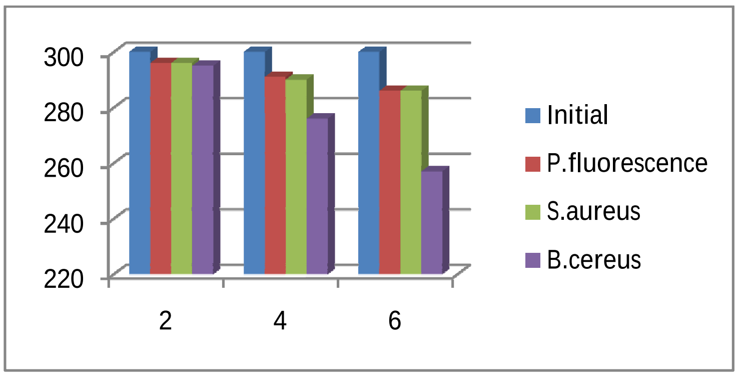
X – axis : time in hours

Y – axis : calcium concentration in ppm

At1% urea concentration



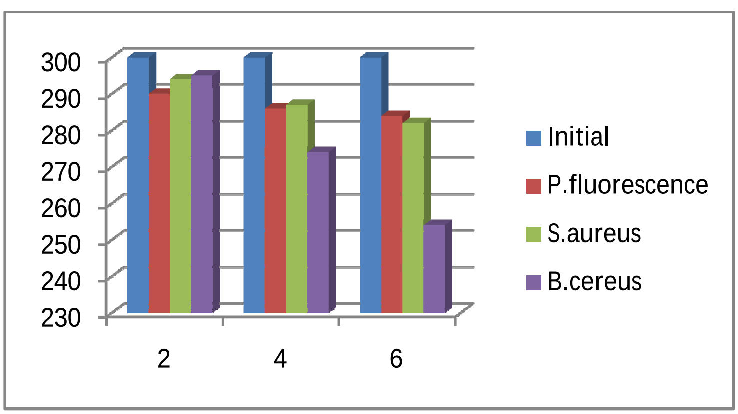
At 2% urea concentration



X – axis : time in hours

Y – axis : calcium concentration in ppm

At 4% urea concentration



X – axis : time in hours

Y – axis : calcium concentration in ppm

CONCLUSION:

The present study revealed that the efficiency of biological removal of calcium might be increased by selection of organisms. The organism also be 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. For the biological calcium reduction in makeup water of cooling water process, three different bacteria were isolated from calcium rich source, Based on ureolytic microbiological calcium carbonate precipitation *Bacillus cereus, Pseudomonas fluorescence and Staphylococcus aureus* and respective methods like batch method, continuous method and immobilization technique. Among these three methods immobilization technique is considered to be effective because of the higher rate of calcium reduction and the stability of P^H.

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