Comparative Study of Bioreduction of Water Containing Hexavalent Chromium Using Biofilm Forming and Non-Biofilm Forming Bacteria

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***Abstract*—Bioreduction is the increase and decrease in the valence state of a metal by metabolically active bacteria. Hexavalent chromium present in waste water is found to be toxic in nature which causes mutation and cancer. This waste water was reduced by using two organisms, a biofilm forming bacteria and a non biofilm forming bacteria. The process of reduction was studied by using analytical methods and growth of the organism was optimized by using different temperature and pH for increasing the bioreduction. It was found that biofilm forming bacteria could reduce more effectively than the bacteria which do not form biofilm.**

Keywords*— *Biofilm; Bioreduction; Hexavalent Chromium.

1. INTRODUCTION

Water is one of the most important natural resources of our planet, in which clean water or domestically usable water availability is getting reduced due to increased waste disposal. Recycling is the best way to salvage this diminishing natural resource, hence regulations concerning wastewater treatment has become more rigorous in the last decade. Remedy for this can be found by the biofilms which are being used more frequently to aid the treatment of wastewater.

Hexavalent chromium [Cr(VI)] is a mutagen and carcinogen it is a cause of significant concern in water and wastewater. A simple and non-hazardous means to remove Cr (VI) is bio-reduction to Cr (III), which is to be precipitated as Cr $(OH)_3$. The bio-reduction required is achieved by Cr (VI) reducing bacterial biofilm that uses hydrogen (H_2) as an electron donor.

Micro-organisms such as bacteria attached to various surfaces such as metals, plastics, soil, etc. get embedded themselves via an extracellular polymeric substances called as EPS (Extra-cellular polysaccharide) by which they form spatially heterogeneous structure collectively called as Biofilms.

A biofilm is any group of microorganism in which cells stick to each other and often these cells adhere to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm extracellular polymeric substance, which is also referred to as slime (although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which by contrast, are single-cells that may float or swim in a liquid medium.

Many numbers of bacterial biofilms can reduce chromium from hexavalent to trivalent state. The capability of Cr (VI) reduction has been found in variety of micro-organisms including strains of *Pseudomonas, Bacillus, Escherichia, Enterobacter, Shewanella, Vogococcus* etc. Enzymes present in the cells of these biofilms are responsible for reduction of Cr (VI) to Cr (III). In our study we have utilized two different organism's one forming biofilm and another non biofilm producer.

2. EXPERIMENTAL DETAILS

2.1 Sub-culture of microorganisms

The 2 isolated organisms were sub-cultured in nutrient broth and were incubated at 37°C for about 6-7 days till the thin layer of biofilm was formed. One of the organisms produced a filmy layer while the other one was unable to produce biofilm. The culture on the biofilm was further sub-cultured on nutrient agar slants and incubated at 35°C, these species were refrigerated at 4^{0}C, ceasing the metabolism of bacterial strains.

2.2 Media Preparation

2.2.1 Nutrient broth

The nutrient medium used for culturing the organisms consisted of 5g of peptone, 3g of beef extract, 5g of NaCl added in one litre of distilled water which was thoroughly stirred and tightened with cotton plug. Further it has been sterilized in autoclave for 20min at 15lb pressure and cooled for 30 min.

2.2.2 Selective media

It is the selective media for isolates which consisted of 20g of

protease peptone, 1.5g of K_2HPO_4, 1.5g of $MgSO_4.7H_2O$, 20g of agar suspended in 1000ml distilled water sterilized for 20min at 15lb pressure.

2.3 Preparation DPC Reagent

The reagent was prepared by dissolving 250 mg of Diphenylcarbazide in 50 ml of Acetone and then adding 50 ml of distilled water.

2.4 Preparation of hexavalent chromium sample

The potassium dichromate was the source of Hexavalent chromium. The chromium sample with concentrations 100ppm, 200ppm, 400ppm, 500ppm, 800ppm, 900ppm, 1000ppm was prepared by dissolving varying concentration potassium dichromate in 100ml of selective medium. Later it was sterilized in autoclave for 20min at 15 lb pressure.

2.5 Hexavalent chromium reduction studies

The 5% of the overnight incubated organisms were inoculated to the 250ml flasks containing selective medium with chromium sample, the flasks were incubated at 37℃ in shaker and the samples were subjected to the estimation of Hexavalent chromium every 24hrs.

2.6 Analytical method

The samples (1.5ml) from the flasks were drawn at 24 hrs time of intervals in pre-weighed eppendorf tubes and centrifuged at 5000rpm for 5min.The supernatant was used to determine the concentration of hexavalent chromium. The concentration of hexavalent chromium is determined spectrophotometrically by the reaction with Diphenylcarbazide.

2.6.1 Diphenylcarbazide assay

100 micro-litre of supernatant was collected in test tubes; 9.9 ml of distilled water was added to set the volume to 10ml. Later 2ml of Diphenylcarbazide was added which reacted with 1-2 drops of conc.HNO_3 indicated by formation of pink color. The intensity of the color formation was greater for sample with higher concentration of chromium which was measured spectrophotometrically at 540 nm.

2.6.2 Biomass estimation

The remaining supernatant from the centrifuged eppendorf tube was discarded carefully without disturbing the pellet. The eppendorf tube with pellet was weighed and the biomass was calculated by deducting weight of empty eppendorf tube.

2.7 Optimization experiments

To enhance the growth of the organism the optimization of the process was carried out. The selected parameters for the purpose were Temperature and pH.

2.7.1 Temperature optimization

The purpose of carrying out this experiment was to analyze the effect of temperature on the growth of organisms and to determine the optimum temperature favorable for the growth of organisms. The 5% of the overnight culture of two isolates (1,2) was inoculated in to the flasks containing 200ml of selective medium and were incubated at 20^0 C, 30^0 C , 35^0 C , 40^0 C temperatures in duplicates and Optical Density (O.D) was measured at 660 nm at the time interval of 24 hrs for 7-8 days.

2.7.2 pH Optimization

The purpose of carrying out this experiment is to analyze the effect of pH on the growth of 2 organisms and to determine the optimum pH favorable for the growth of organisms. The 5% of the overnight culture of two isolates (1,2) was inoculated in to the flasks containing 200ml of Selective medium with varying pH (5, 6, 7, 8, 9) in duplicates and O.D was measured at 660nm at the time interval of 24hrs for 7-8 days.

The standard graphs of O.D vs. No of days for different temperature and pH was plotted to determine the optimum temperature and pH ideal for the efficient growth of organisms.

2.7.3 Reduction analysis with the formation of biofilms

To adhere the microorganisms 6cmx6cm of nylon mesh was used. 150ml of Selective media was taken in the 6 culture bottles in duplicates(i.e., 2 controls with biofilm, 2 test of pH 5 with biofilm and 2 test of pH 6 with biofilm)for both the isolates(1,2), the culture bottles were incubated at the temperature 35° C . The O.D was measured at 66Onm and the DPC assay was done for the tests with pH 5 and 6.

3 RESULTS AND DISCUSSION

The bacterial strains isolated from the tannery effluent are checked for hexavalent chromium tolerance with a different concentration of $K_2Cr_2O_7$ 100ppm, 200ppm, 400ppm, 500ppm, 800ppm, 900ppm, 1000ppm). The reduction of Hexavalent chromium was analyzed by DPC test at 540 nm for 11 days.

The figure (1) illustrates the reduction of Hexavalent Chromium at 540 nm up to 11 days. It was observed that the two organisms efficiently reduced Hexavalent Chromium for about 11 days and later the rate of reduction remained constant. Among these two isolates one of them was able to produce biofilm while other one could not produce biofilm. The optimization of the process for the growth of organisms was carried out for a various pH and temperatures.

The figure (2) illustrates the reduction of the Hexavalent Chromium at 540nm up to 11 days.

The figure (3) illustrates the growth of the organisms for temperatures ^{0}C (20, 30, 35, and 40). The O.D. at 660 nm showed the maximum growth for temperature 35⁰C.

The figure (4) illustrates the growth of the organisms for pH (5, 6, 7, 8, and 9). The O.D. at 660 nm showed the maximum growth for pH 6.

The figure (5) illustrates the reduction of hexavalent chromium with biofilms at optimum process conditions (pH-6 and Temperature 35⁰C).The maximum reduction of

hexavalent chromium at pH 5 and 6 was 48.92 % and 56.23% respectively.

The figure (6) illustrates the comparison between reduction of 1000ppm of hexavalent chromium with biofilms and without biofilms, it was inferred that by the formation of biofilms the reduction of hexavalent chromium increased about 16%.

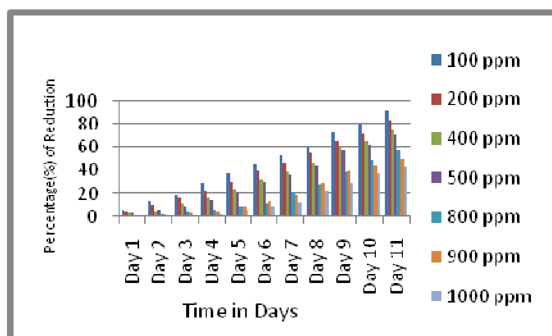

Figure 1 Reduction analysis without biofilm formation (%).

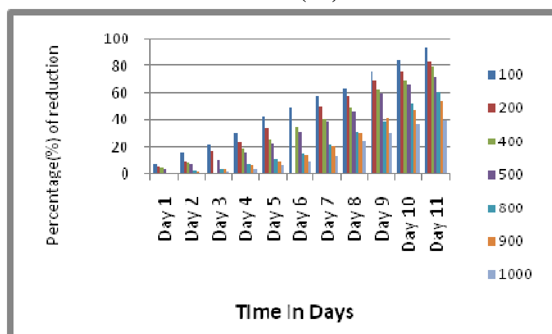

Figure 2 Reduction analysis with biofilm formation (before optimization)

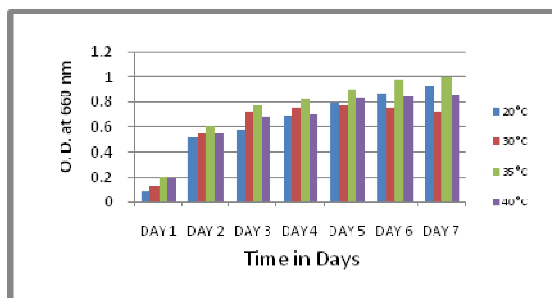

Figure 3 Temperature optimization

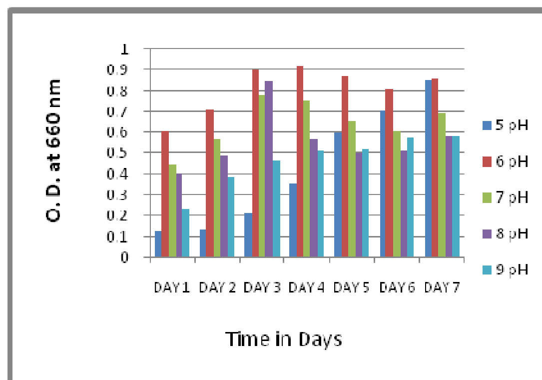

Figure 4 pH optimization

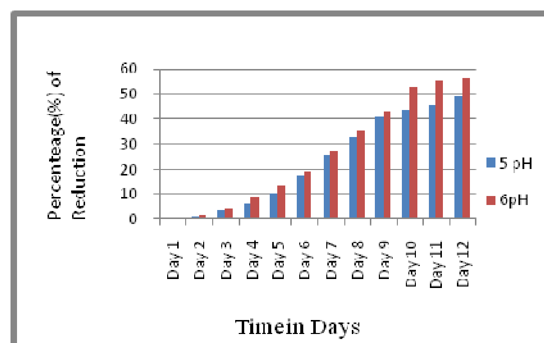

Figure 5 Percentage reduction of Cr (VI) with Biofilm formation

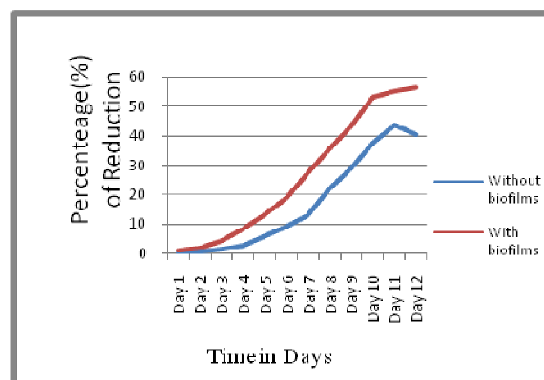

Figure 6 Assessment of reduction efficiency with biofilms for 1000ppm of Hexavalent Chromium

CONCLUSIONS

Bioreduction of hexavalent chromium in the waste water was found to be efficient method of reducing the toxic form of hexavalent chromium to non toxic trivalent chromium.

The growth of the organisms was optimized with different pH and temperature, and the optimum pH of 6 and temperature of 35^0 C was found to be the conditions required for growth and reduction process.

The maximum reduction for 1000ppm of hexavalent chromium was about 40.26% by non-biofilm forming bacteria and in the presence of biofilms the organisms reduced the hexavalent chromium to about 56.23%.This shows that the reduction of the biofilm forming organisms was about 16% more efficient than the non- biofilm forming organism.

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