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Biodegradation of heavy metal contaminated soil and optimization of heavy metal degradation

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Abstract

Heavy-metal pollution plays an environmental problem due to the toxic effects of metals. The present study was to identify the potential bacteria as the bioremediation agent. Bacteria isolated through spread plate method and identified through 16s rRNA sequencing. Media was optimized by following effects such as concentration of metals, temperature, pH, carbon source, nitrogen source. Selected isolates were screened through 0.1% Pd, Cr and Cd on minimal salt agar medium. Bacteria were identified as *Bacillus cereus*. Highest degradation was recorded up to 94% optimized through optimization of various effects. As a result, this proves that the bacteria present at soil sample of coalfield are important to bioremediation activity to reduce the heavy metal pollution in the environment.

Keywords: heavy metal, bioremediation, optimization, degradation, *Bacillus cereus*

Introduction

Microorganisms are very huge group of single-cell and consist of extensive variety of niches of occurrence, metabolic types, environmental habitats and geometric shape. Microorganisms can live in all biospheres such as soil, water, ocean floor, hot springs, and the Earth’s crust. Some microorganisms are pathogenic that can be cause diseases but some microorganisms are needed to useful in bioremediation activities. (Ragini, G. and Pankaj, B. 2012).

Bioremediation is the process to break down or transformation of dangerous materials to harmless compounds (Mandal, A. K. *et al*., 2012). This technique is much effective than other techniques (Alavijeh, P. K *et al*., 2014). The bioremediation method is cheaper are used to cleanup of hazardous waste. (Naik, M. M *et al*., 2012) It is environment friendly too and very useful to eliminate pollutants (Sharma, S. 2012).

Water and Soil are contained a diversity of microorganisms such as bacteria. Particularly, they adapted high levels of toxic metals from environments contaminants and that it’s useful for bioremediation applications (Salem, I. B *et al*., 2012). The bacteria are essential in dietary chains and plays important roles in natural equilibrium. *Bacillus, Staphylococcus, Methylococcus Micrococcus and Pseudomonas* to degrade different types of contaminants in different matrixes such as water, soil, air and sediments has been generally acknowledged (Bahig A. E. *et al*., 2008).

In environment there are a variety of metal contaminants like antimony, arsenic, beryllium, chromium, cadmium, copper, mercury, lead, iron, magnesium, manganese, radium, selenium, silver, nickel, thallium and zinc present in surface groundwater, water, soil, industrial gaseous emissions (Perriello. 2005). The familiar heavy metal bioremediation bacteria are such as *Escherichia coli, Bacillus licheniformis, Salmonella typhi, and Pseudomona fluorescence* (Basha, S.A. and Rajaganesh, K. 2014), *Pseudomonas aeruginosa, Rhodobium marinum, Alcaligenes eutrophus, Enterobacter cloaceae,* and *Rhodobacter sphaeroide* (Garbisu, C. and Alkorta, I. 2003, Dash, H.R *et al*., 2012).

So present study was investigated the isolation and identification of coal mine bacteria capable in degradation of heavy metals (Pb, Cr and Cd) and were optimized the condition of heavy metal degradation.

Material and Methodology

Collection of Sample

Soil Samples were collected in sterile glass – screw cap tubes from Jharia coal mine Dhanbad, Jharkhand.

Isolation, Screening and Identification of bacteria

The heavy metal degrading bacteria were isolated from coal mine soil sample by serial dilution technique on nutrient agar medium and bacteria were screened on minimal salt agar medium with 0.1% concentration of Pb, Cr and Cd. (Azad A.K *et al*., 2013) The selected isolates were identified through Biochemical test followed by Bergey’s Manual and 16s rRNA sequence.(Carlos, F.S *et al*., 2016)

16s rRNA sequence determination

The bacterial strain was grown overnight in Nutrient media medium and the genomic DNA was isolated (Mak and Ho, 1991). Bacterial 16S rDNA was amplified from the extracted genomic DNA by using the following universal bacterial 16S rDNA primers forward primer (5' AGAGTTTGATCMTGGCTCAG 3') and reverse primer (5' TACGGYTACCTTGTTACGACTT 3'). PCR reactions for the detection of the bacterial species were set up in a clean room with pipettes reserved specifically for this purpose. The PCR reactions were performed in 20 µl volumes containing 2 µl of the genomic DNA sample, 1× PCR buffer containing; 0.16 mM dNTP Mix; 20 pmol of forward and reverse primers and 0.75 U Taq DNA polymerase (MBI, Fermentas, Lithuania). The mixes were overlaid with 2 drops of mineral oil. Amplification was carried out in a thermal cycler (Eppendorf Mastercycler nexus 230 V/50-60 Hz) with the PCR conditions as follows: an initial denaturation at 95°C for 6 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 1 min, and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min. PCR products were analyzed using 2% agarose gel electrophoresis. Sizes of the amplified 16S rDNA segments using primers were estimated using molecular weight markers NEX-GEN DNA Ladder (100 bp) and the gel was run at 100 volt for 45 minutes at room temperature. The PCR products was stained with ethidium bromide and visualized on Gel Documentation System (Zenith, Gel Documentation System; Model No. Gel.LUMINAX-312).The samples were then sequenced using Sanger’s Method.

Sequence alignment and data analyses

The obtained forward and reverse sequences were aligned using online pairwise alignment tool BioEdit. The query sequences were identified considering E value a $<1 \times 10^{-5}$ and maximum hits (99 or 100%) with a species in the reference database NCBI. In addition to BLAST, MEGA X was used for phylogenetic tree analysis employing Neighbour Joining (NJ) method

Optimization

The selected bacteria were used to optimize highest degradation of heavy metals. Optimization effect such as concentration of metals, temperature, pH, carbon source, nitrogen source of heavy metals were optimized to achieve highest degradation. (Zahoor, A.; Rehman, A, 2009)

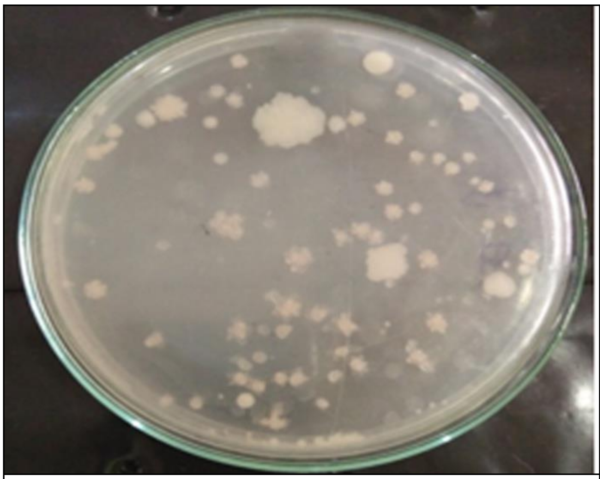
Fourier transform infrared spectrometric analysis

Fourier transform infrared spectrometric (FTIR) was used to identify the functional groups presence on the cell surface which was responsible for the adsorption of heavy metals. The absorbance of the IR spectrum was recorded within 500 to 4000 cm^{-1} using a FTIR (François, F. *et al*., 2012).

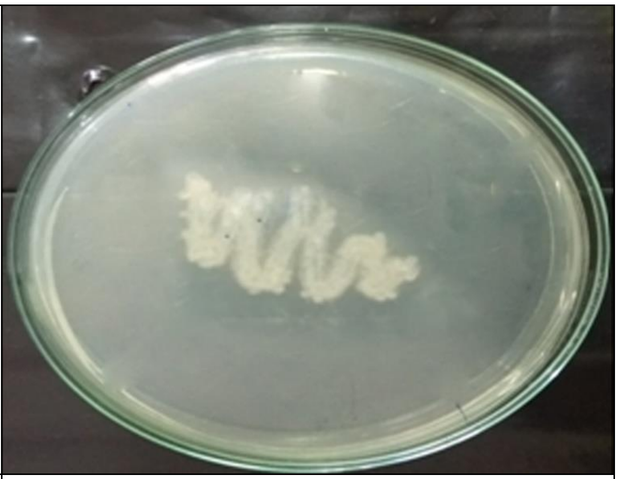
Result and Discussion

Isolation and Screening of heavy metal resistant bacteria

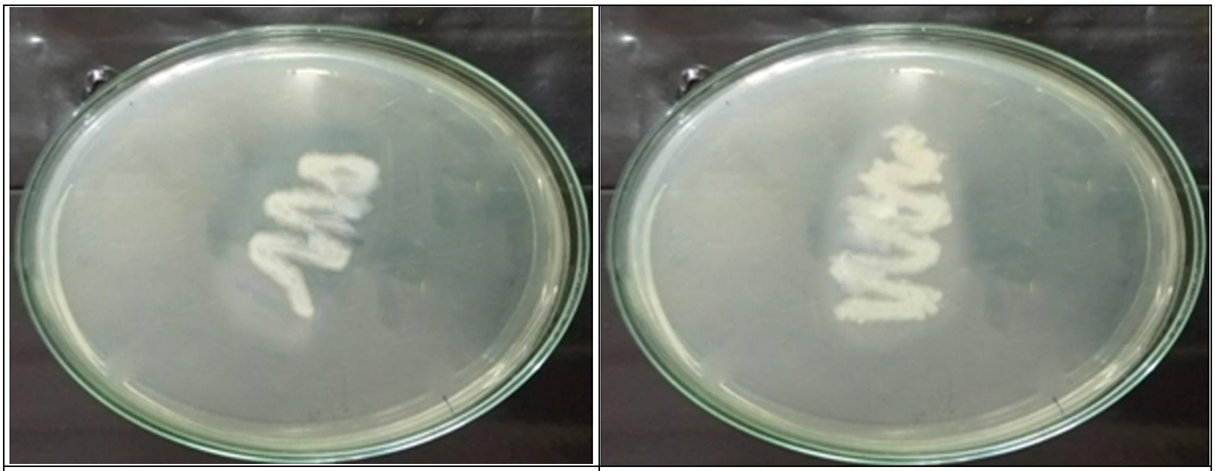
The one selected isolate was showed positive results against Pd, Cr and Cd. The selected isolate was identified through biochemical test and 16s rRNA Sequencing as *Bacillus cereus.*



Isolated Bacterial colony from soil



Screening of bacteria (Pd)

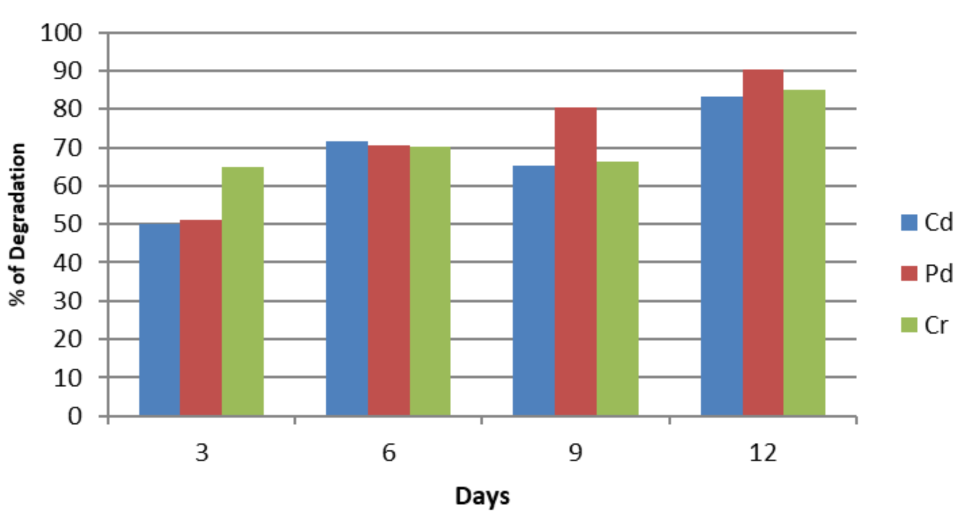


Screening of bacteria (Cr) Screening of bacteria (Cd)

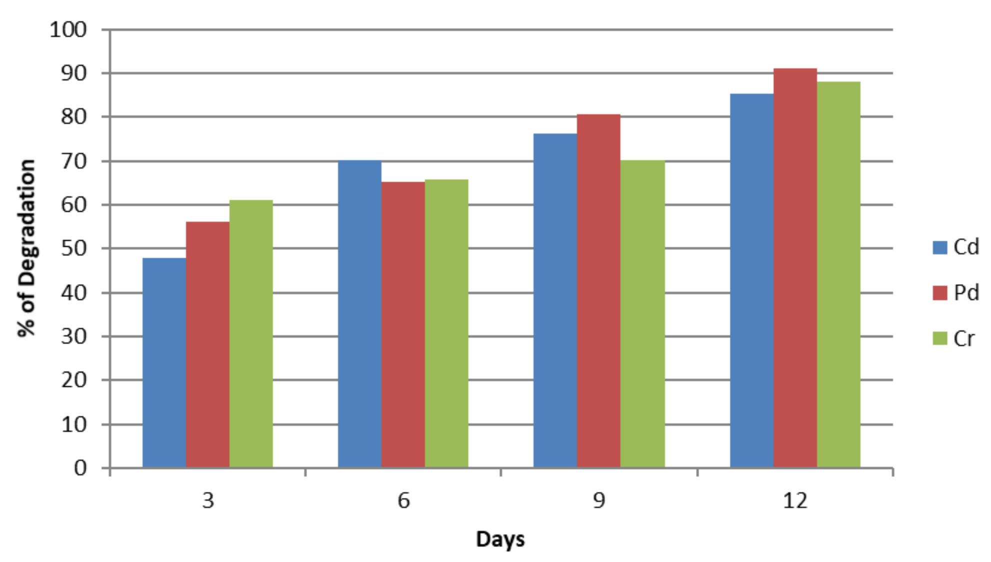
Fig 1

Optimization Parameters

The effective degradation of Pd, Cr and Cd were made along with the high and low amount of pH, Concentration of metals, Temperature, carbon source and nitrogen source. The pH taken various range from 2 to 10. At pH 8 the degradation recorded higher whereas at pH 2 and pH 10 showed maximum degradation. (Graph 1) Below range of pH 7 degradation recorded about 64% and at pH 10, recorded degradation about 70%.



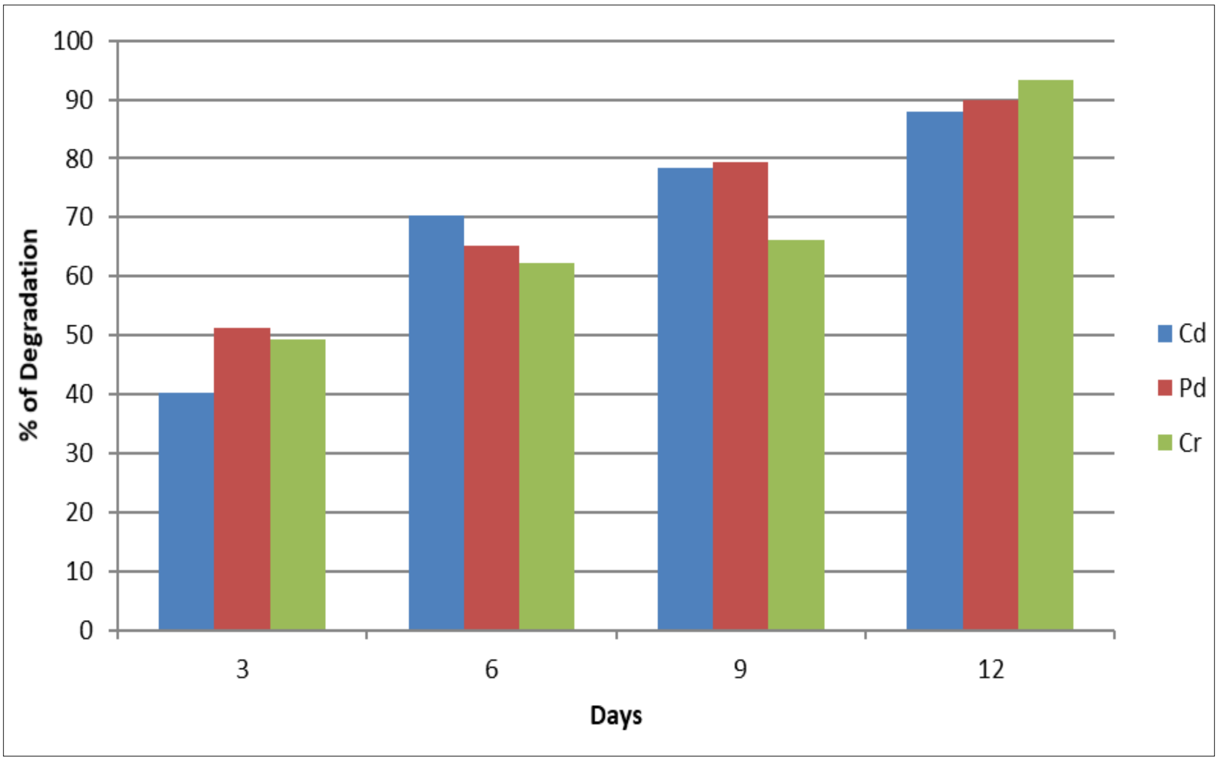
Graph 1: Optimization of Cr, Pd and Cd at pH 8



Graph 2: Optimization of Pd, Cr and Cd at 35^0 C

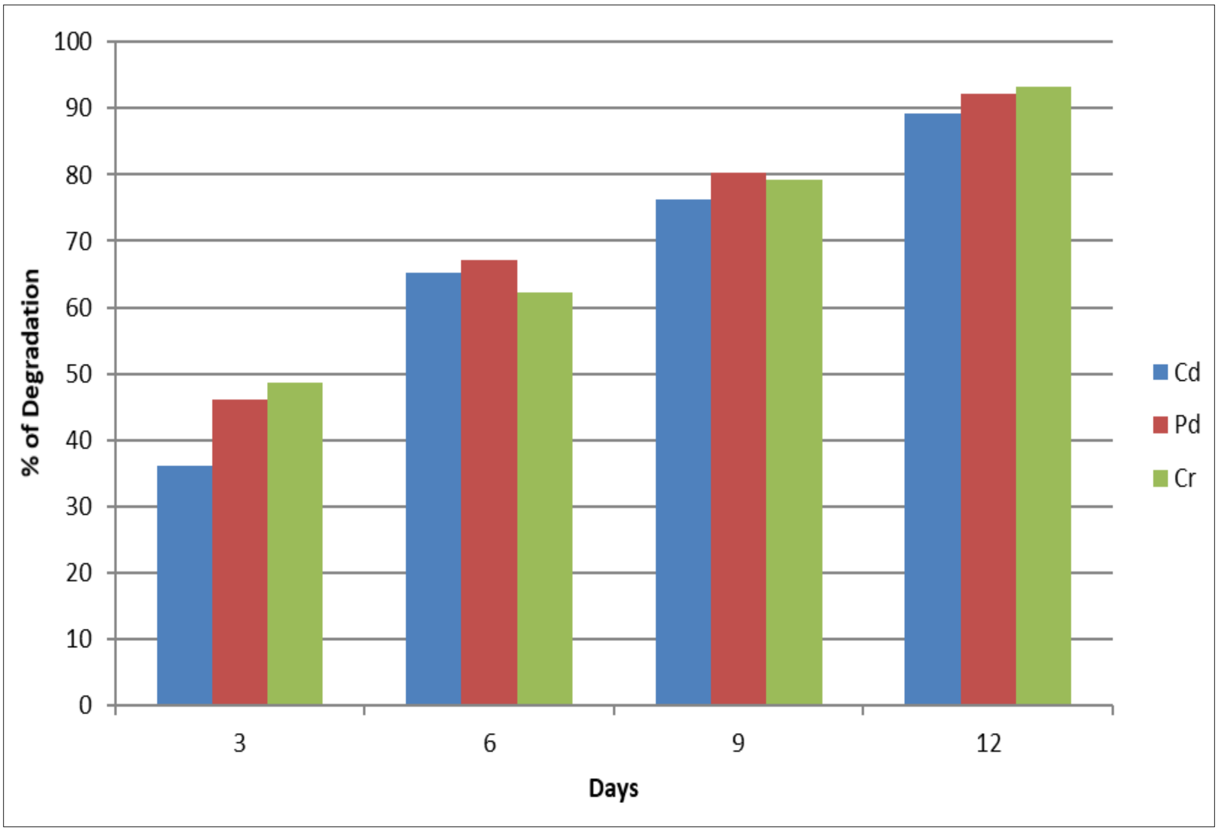
At different temperatures highest degradation was observed at 35^0C while below 30^0C showed degradation about 58.8% and above 40^0C recorded degradation 69%. (Graph 2)

In Yeast extract the highest degradation of Pd, Cr and Cd were done at 0.3% in *Bacillus cereus* (Graph 3) and below 63.5% degradation was recorded at various concentrations (0.1, 0.2, 0.4 and 0.5%).



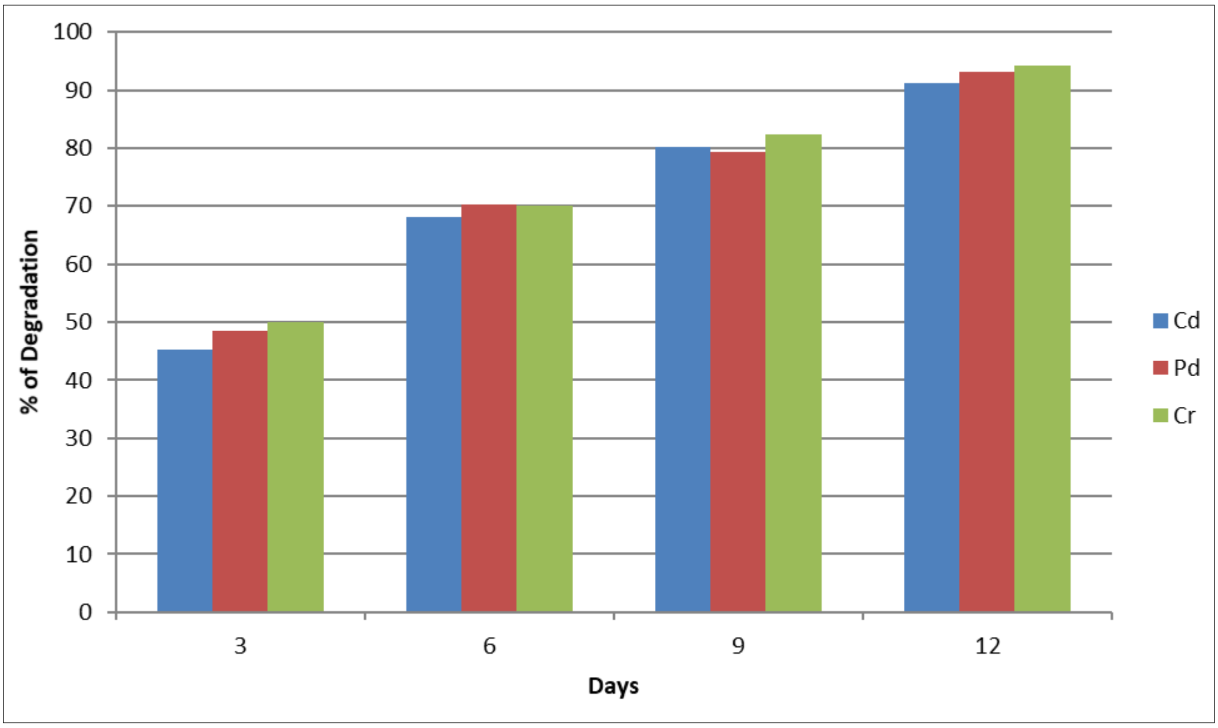
Graph 3: Optimization of Pd, Cr and Cd at 0.3% of yeast extract

In Glucose the highest degradation of Pd, Cr, and Cd were done at concentration of 0.1% in *Bacillus cereus* (Graph 4) and the degradation rate was below 70.3% at other concentrations (0.2, 0.3, 0.4 and 0.5%).



Graph 4: Optimization of Pd, Cr and Cd at 0.1% of Glucose

In ammonium sulphate the highly degradation of Pd, Cr, and Cd were done at concentration 0.1 in *Bacillus cereus* (Graph 5) and the degradation rate was below 65.3% at other concentrations (0.2, 0.3, 0.4 and 0.5%).



Graph 5: Optimization of Pd, Cr and Cd at 0.1% Ammonium sulphate

FTIR Analysis

FTIR spectroscopy analysis was performed to identify the functional groups that played an important role in the method of biosorption of Pd, Cd and chromium. FTIR analysis of Pd, Cd and chromium exposed biomass contained some absorption bands at various wavelengths. Which indicated the different functional groups like bonded and non-bonded hydroxyl (O-H) groups, an amine (-NH2), amide (-CONH-), aromatic (C = C) ring, carboxyl (-COOH), aliphatic (-CH2) groups, alkenes (C = C) etc. High absorption bands were recorded at 3299.65 cm^{-1}. Which was indicates the presence of bonded or the non-bonded hydroxyl group (O-H) as well as amine N-H stretching in that region.

Conclusion

In conclusion, present study investigated that bacteria isolated from contaminated soil sample identified as *Bacillus cereus* and shows positive result in bioremediation process and optimized the media condition for highest degradation of heavy metals. Therefore, it can be utilized as potential bioremediation agent to eliminate the heavy metal pollutant in future.

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