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HEAVY METAL RESPONSE BEHAVIORS OF SULFUR-OXIDIZING *Pseudomonas* sp. PRK786

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ABSTRACT

Heavy metals (Mercury, Lead, Chromium and Bismuth) response behaviors (Freundlich model) of indigenous *Pseudomonas* sp. PRK786 was analyzed using thiosulphate medium supplemented with 5% of sodium thiosulphate. Monitored the biomass against the control and the absorbance values at OD_{440} (Test-0.983 and control- 1.23 (0.5µg *E.coli*/ml)) were converted to dry weight (0.399 µg of SOB/ml). The behaviors like growth, pH, conductivity, total protein content, total thiosulphate, and sulphate ion concentration of heavy metal treated culture (*Pseudomonas* sp. PRK786) were significantly inhibited in the following order: Bismuth<Chromium<Lead<Mercury. From the results revealed, that the mercury (95%-10ppm) and lead (92%-10ppm) were completely inhibits the all biochemical behaviors than thechromium (50%-20ppm) and bismuth (10%-20ppm). The FTIR analysis of bioadsorption of heavy metals on the bacterial surfaces results suggested that, the absorption bands around $1400Cm^{-1}$ of all treated bacterial surfaces indicates adsorption sites of on the heavy metalsexcept in untreated bacterial surface (Mercury – $1400.63Cm^{-1}$, lead – $1403.76Cm^{-1}$, Chromium – $1403.76Cm^{-1}$, Bismuth – $1402.28\ Cm^{-1}$). The absorption bands around$1400cm^{-1}$ is due to the vibration of C=O of COO- carboxylate ions and O–H carboxylate ions are may be responsible for adsorption of these heavy metals.

Key words:*Pseudomonas sp.* PRK786, thiosulphate, biomass, absorbance, and FTIR.

INTRODUCTION

Both directly and indirectly discharge of heavy metals (lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper, and nickel) from fertilizer, metallurgy, petroleum, electrolysis, leather, printing, paint, electronic manufactures, power plants and batteries manufacture industries into aquatic ecosystems. It became a matter of concern in

India over the last few decades and it has created a life threating problems [1 & 2]. There is considerable interest was raised for microbiological processes can affect the behavior of metal contaminants in natural and engineered environments and their potential for bioremediation [3]. Biosorption of heavy metals by *Pseudomonas* from wastewater [4], Soil [5], and aqueous solution [6] were studied. Mercury is a very toxic element that is widely spread in the atmosphere, lithosphere, and surface water. Concentrated mercury poses serious problems to human health, as bioaccumulation of mercury within the brain and kidneys ultimately leads to neurological diseases. To control mercury pollution and reduce mercury damage to human health, sensitive determination of mercury is important, suggesting that sulphur oxidizing bacteria (SOB) may provide revolutionary tools in biomedical and environmental monitoring of mercury [7] and *pseudomonas aeruginosa*[8].The previous study evaluates the biosorption of Cr (VI) by *pseudomonas aeruginosa*[9] andtannery effluent inhabitant *Pseudomonas* spp.[10]. Biosorption of heavy metals like Zn (II), Cu (II), and the binary mixture of these two metal ions by the indigenous *Thiobacillusthiooxidans*was investigated [11]. Biosorption of lead from aqueous solutions by soil born *Pseudomonas*sp was studied [12]. A technologically and economically feasible process called bioleaching was used for the removal of heavy metals from livestock sludge with indigenous sulfur-oxidizing bacteria [13]. Sulphur Oxidizing Bacteria (SOB) is a group of microorganisms widely used for the biofiltration[14]. Main objectives of this study were to; determination of dry weight, analyses the heavy metal response behaviors like growth, pH, conductivity, total protein content, total thiosulphate, sulphate ion concentration of culture and bioadsorption of heavy metals in bacterial surface by FTIR.

MATERIALS AND METHODS

Bacteria and growth conditions

Thiosulphate medium were employed by enriching with (5% of sodium thiosulphate) for cultivation of sulphuroxidizing *Pseudomonas* sp. PRK786.The medium contains 5.0 g $Na_2S_2O_3$, 0.1 g K_2HPO_4, 0.2 g $NaHCO_3$, 0.1 g NH_4Cl in 1000 ml distilled waterandbromocresol purple (pH indicator) with pH 8.0. In addition, 5.0 g of glucose, 0.5g of yeast extract and 0.5g of peptone were added /per liter.Inoculate the *Pseudomonas* sp. PRK786 form stock into medium and incubated at 37^{o}C for 24 hrs. Cells were grown aerobically with CO_2 (by 0.2 g $NaHCO_3$/100ml) enriched and harvested in the late exponential growth phase (D_{440} between 0.240 and 0.250) by centrifugation at 10,000 g at 4 ^{o}C.

Determination of biomass

Cells harvested from an exponential phase by centrifugation at 5000rpm for 5 min, pellet was washed twice with 33 mMTris/HCl buffer (pH 8.0) and resuspended in 50 mMformate buffer, pH 3.0. Enabling absorbance at 440 nm used for monitored the biomass: Absorbance values were converted to dry weight by reference to a calibration curve prepared for suspensions of organisms (0.5µg *E.coli*/ml) dried to constant weight at 105 ^{0}C using ovan.[14]

Assay the Heavy metal response behaviors

Heavy metal containing sterilized thiosulphate medium were prepared using different concentrations of mercury chloride, lead acetate, bismuth sulphate and potassium dichromate (in the form of hexavalent chromium - supplied in the form of potassium dichromate solution in deionized water and filter-sterilized before use) solution ranging from 5 to 20 mg/l (5-20ppm).Lag phase culture was inoculated flowed by incubated for 37^{o}C for 24 hrs with orbital shaking (120rpm), after incubation period; cells were harvested by centrifugation at 6000 rpm for 15 min at 4°C.

The growth of *Pseudomonas* sp. PRK786 was monitored by absorbance (UV–VIS Spectrophotometer UV-1700- Shimadzu)at OD_{600nm} =1. The pH and conductivity of the culture supernatant were measured using a digital pH meter (elico-LI120) with regular intervals. Before, the estimation of protein (Lowry's method), culture were centrifuged, washed with distilled water and finally heated in a boiling water bath for 10 min after adding 2.5 ml 0.5M NaOH for solubilized protein read at 670nm. Changes in thiosulphate concentrations were measured by the decoloration of methylene- blue at 670 nm in acidic conditions,and the changes in SO_4^{2-} were measured by using $Bacl_2$ precipitation method. The pellets were washed thrice with deionized water to remove heavy metals not absorbed by the bacteria it is used for FTIR analysis[16, 17, 18]

Bioadsorption of heavy metals on the surface SOB by FTIR[a].

The bacteria has pelleted by centrifugation of 50 ml cultures at 6000 rpm for 15 min at 4°C and subjected into vacuum desiccated using an ROCKER vacuum bump. Heavy metals loaded biomass was washed dried and powdered after bioadsorptionof heavy metal ions under the same conditions. One milligram of finely crushed biomass was mixed with 400 mg potassium bromide. The mixture was ground into fine powder and translucent sample disks obtained by using a manual hydraulic press at a pressure of 100 kg cm–2 for 10 min. The disk was fixed in an FTIR[a] Spectrophotometer (FTIR 8400S SHIMADZU). FTIR spectrum of the biomass unexposed (control) and exposed to heavy metals at concentration of 15 mg/l were obtained from 500 to 4000 cm–1.[9]

RESULTS AND DISCUSSION

Bacteria and growth conditions

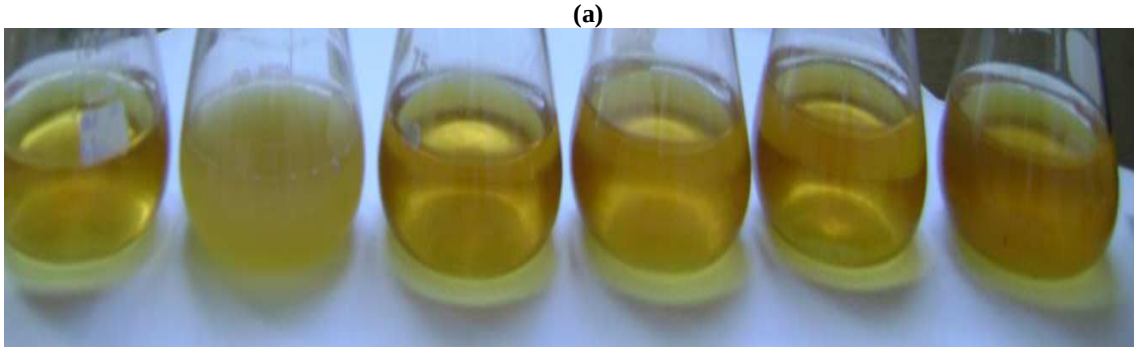
Fig.1 shows from left conical flash has thiosulphate medium with bromocresol purple (control) indicate no growth (purple color retained) and right flash was loses the purple color due to growth of *Pseudomonas* sp. PRK786. (D_{440} between 1.740 OD, pH – 1.3 (responsible for loses of purple color of the medium), conductivity (emf) – 350mV, protein concentration – 2.3µg/ml were observed at 37°C for 21 hrs.

Fig.1 Growth of sulphur oxidizing bacteria in thiosulphatebrot

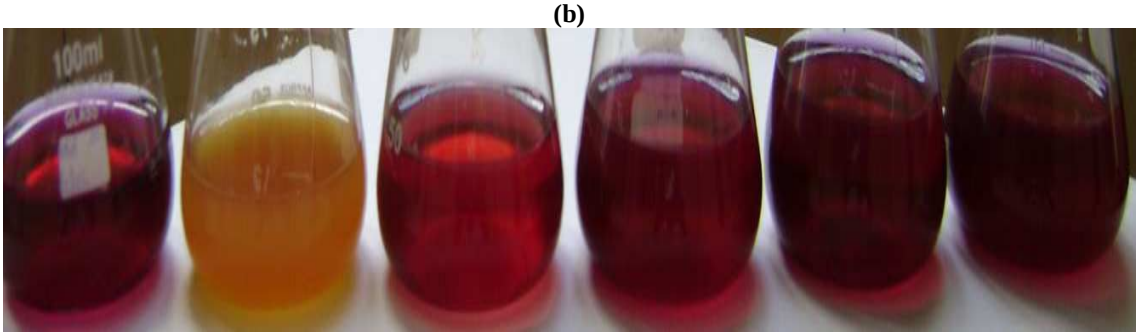
Dry weight

Washed pellets were resuspended in 3ml of 50 mMformate buffer, pH 3.0 dry cell weight measurement was carried out by OD at 440nm - 0.983 and the control (0.5µg E.coli/ml) OD is 1.23 that will express relative biomass in the suspended solution. Absorbance values were converted to dry weight (0.399 µg of SOB/ml) against the control dry weight. In addition to rechecked dry biomass of the organisms dried under constant at 105 ^{0}C using muffle burner. Dried biomass (0.274 µg) is slightly changing then the relative biomass. A report has been said to us a predetermined concentration of the indigenous *T. thiooxidans*biomass (0.3 g of dry cell weight/ml) was used to study the biosorption of Zn(II) and Cu(II) [11].Biomass was detailed reported in [15] Biomass production increased with increasing substrate concentrations: thus the yield increased from 5.5g dry wt/mol^{-1} for thiosulphate, 7.0g dry wt/mol^{-1} for trithionate and 12.5 g dry wt/mol^{-1} for tetrathionate

(a)



(b)



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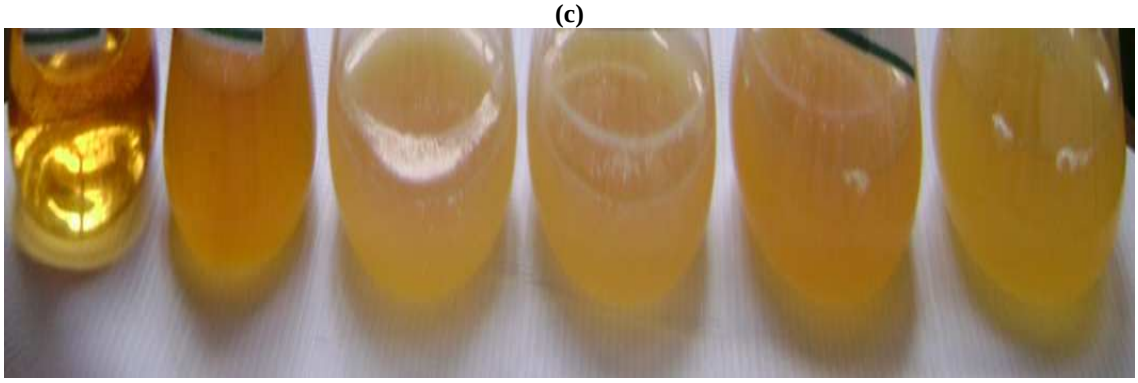
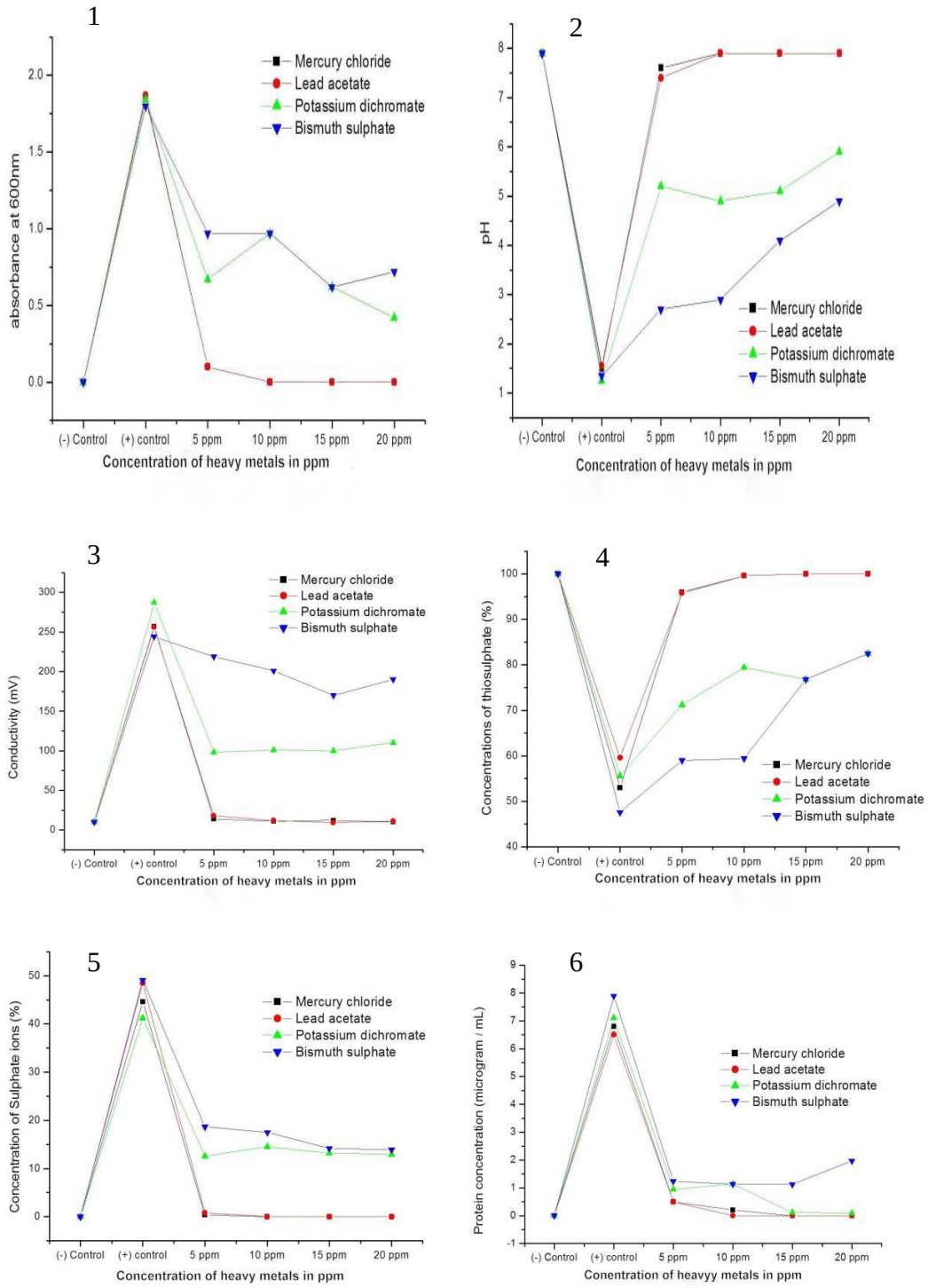


(d)

Fig.2 (a) Existence of Different concentration of mercury chloride treated cultures (*Pseudomonas sp*. PRK786); L to R: negative control, positive control, 5ppm, 10ppm, 15ppm, and 20 ppm of $HgCl_2$treated. **(b)** Existence of Different concentration of lead acetate treated cultures; L to R: negative control, positive control, 5ppm, 10ppm, 15ppm and 20 ppm of $Pb(C_2H_3O_2)_2$treated. **(c)** Existence of Different concentration of potassium dichromate treated cultures; L to R: negative control, positive control, 5ppm, 10ppm, 15ppm and 20 ppm of $K_2Cr_2O_7$treated. **(d)** Existence of Different concentration of bismuth sulphate treated cultures; L to R: negative control, positive control, 5ppm, 10ppm, 15ppm and 20 ppm of $Bi_2O_{12}S_3$treated.



Graph .1 Effects of heavy metal response behaviors of *Pseudomonas sp.* PRK786; **(1)** Absorbance at 600nm, **(2)** pH, **(3)** Conductivity(mV), **(4)** Amount of thiosulphate consumption, **(5)** Amount of sulphate production and **(6)** protein concentration.

Assay the Heavy metal response behaviors

This is a first report to heavy metal response behaviorsof SOB are exhibits valuable results. According to the metals treatment; generally, biosorption of metal ions usually classified as two types the Freundlich model, in which the amount of metal uptake by the biomass increases with time, and the Langmuir model, in which the amount of metal uptake by the biomass reaches equilibrium[19]. Evaluated the heavy metal response of the indigenous *Pseudomonas sp.* PRK786 according to the Freundlich model was tested againstmercury, lead, chromiumand bismuth. From the results, the mercury chloride treated culture significantly reduce their growth even 5ppm concentration shown Graph.1 (1). (absorbanceat OD_{600nm}=0.10). That might be due the inhibition of sulphur oxidizing enzymes (SOE) or glucose catabolic enzyme like membrane-bound glucose dehydrogenase (mGDH) of SOB. Pervious results also coined the same [20]. In addition to that SOB was used thiosulphate as an energy source (because it is chemolithoheterotrophic) and lead acetate treated culture was also observed no growth (absorbanceat OD_{600nm}=0.10) becauseit might also inhibited the SOE andmGDH, since that mode of inhibition was still unknown. Hence, the response against another two (Bismuth sulphate and Potassium dichromate) are less sensitive then the mercury and lead treated culture. Bismuth sulphate and potassium dichromate treated culture was observed a little growth (absorbanceat OD600nm=0.67 and 0.97) respectively. Bismuth and chromium ions are may be involved crabbmling of enzymes activity.

The pH reduction of these cultures was shown in Graph.1 (2), that exhibit the bismuth sulphate treated culture has greatly reduce pH (2.7-5ppm, 2.9-10ppm, 4.1-15ppm, 4.9-20ppm) less greater then the potassium dichromate treated culture pH (5.2-5ppm, 4.9-10ppm, 5.1-15ppm, 5.9-20ppm). The mercury chloride and lead acetate treated cultures; 7.6-5ppm, 7.9-10ppm, 7.9-15ppm, 7.9-20 ppm and 7.4 -5ppm, 7.9-10ppm, 7.9-15ppm, 7.9-20ppm respectively. Finally, bismuth and chromium metal has partially to influence the thiosulphate oxidation due to the crabbmling of thiosulphate oxidizing enzymes.

Variation of conductivity in different conc. of heavy metals treated growing cultures was shown Graph.1 (3), that indicates bismuth sulphate treated culture has observed high conductivity (219mV-5ppm, 201mV -10ppm, 170mV -15ppm, 190mV -20ppm) when the concentration of heavy metal increases the conductivity of the solution was inversely reduced (~10mV) due to the unknown reasons. The same results were observed on the potassium dichromate treated culture (098mV-5ppm, 101mV -10ppm, 100mV -15ppm, 110mV -20ppm) here the reduction rate is ~3.5mV. Hence, mercury chloride and lead acetate treated culture has no change the conductivity that was generated by due to the complete inhibition of growth.

In general, SOB are oxidizing the reduced sulphur compounds especially thiosulphate, it was oxidized into to form undetermined long chain sulphur by thiosulphate oxidizing enzymes: namely thiosulphate dehydrogenase, thiosulphatereductase, tetrathionate hydrolase and sulphiteoxygenase. The groups of enzymes were responsible for thiosulphate oxidation. Here, the growing cultures were treated with mercury chloride, lead acetate, potassium dichromate and bismuth sulphate metals and determined the thiosulphate consumption (%) after 24hrs at 37^0C. From the results shown in Graph.1 (4) mercury chloride and lead acetate treated culture containing SOB do not uptake the thiosulphate as an energy source. They can exist in the medium even the 5ppmconcentration. The following percentage of thiosulphate can exist in medium 96-5ppm, 99.6 -10ppm, 100 -15ppm, 100-20ppm and 95.8-5ppm, 99.6 -10ppm, 100 -15ppm, 100-20ppm respectively. That represents

the above heavy metals are directly interact with thiosulphate consumption. However, untreated cultures containing SOB consume the (53% and 59.9%) respectively. The potassium dichromate and bismuth sulphatetreated cultures contains 71.2-5ppm, 79.4-10ppm, 76.8-15ppm, 82.5-20ppm and 59-5ppm, 59.4-10ppm, 76.8-15ppm, 82.5-20ppm percentage of thiosulphate can exist respectively. Compared then potassium dichromate and bismuth sulphatetreated cultures, the bismuth sulphatetreated cultures has significantly consume the thiosulphate nearly 59%. It is equal to positive control culture. The results shown in Graph.1 (5) indicate sulphate ions production. Protein concentration of heavy metal treated cultures were displayed in Graph.1 (6) only the bismuth sulphate treated culture containing µg/ml of proteins (1.24-5ppm, 1.14-10ppm, 1.12-15ppm, 1.97-20ppm) apart form that other cultures contains no significant amount of protein was observed. Protein concentration of culture mainly depends on the growth.

FTIR

Bacterial surfaces characteristic of SOB (*Pseudomonas sp. PRK786)* by FTIR analysis with and without adsorbed (passively) mercury, lead, bismuth, and chromium were shown in Fig. 3 4, 5, 6 & 7 respectively. A number of absorption peaks in the control sample Fig.3 indicated the completebacterial surfaces characteristics. Shows that the band's observed around at 3415.42 cm^{-1} to 3427.86 cm^{-1} results (form – NH_2) asymmetric stretching mode of amines, which is slightly broad indicating overlapping of amines and hydroxyacyl stretching on the bacterial surface.

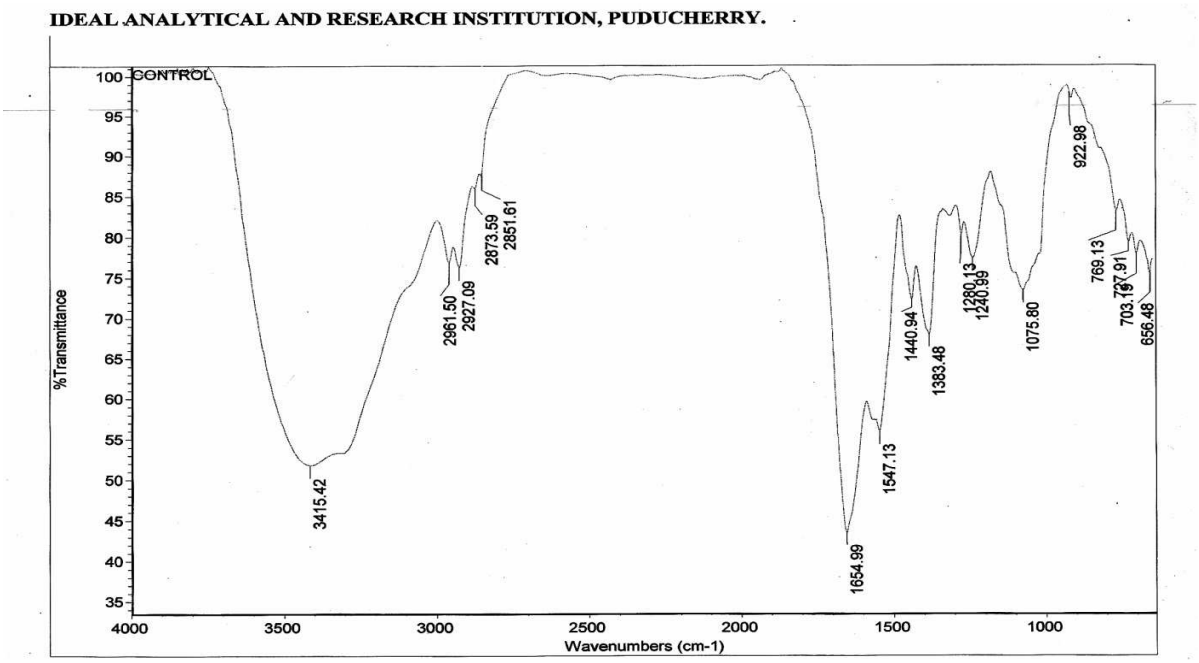


Fig.3 FTIR spectra of *Pseudomonas* sp. *PRK786* surface (Control)

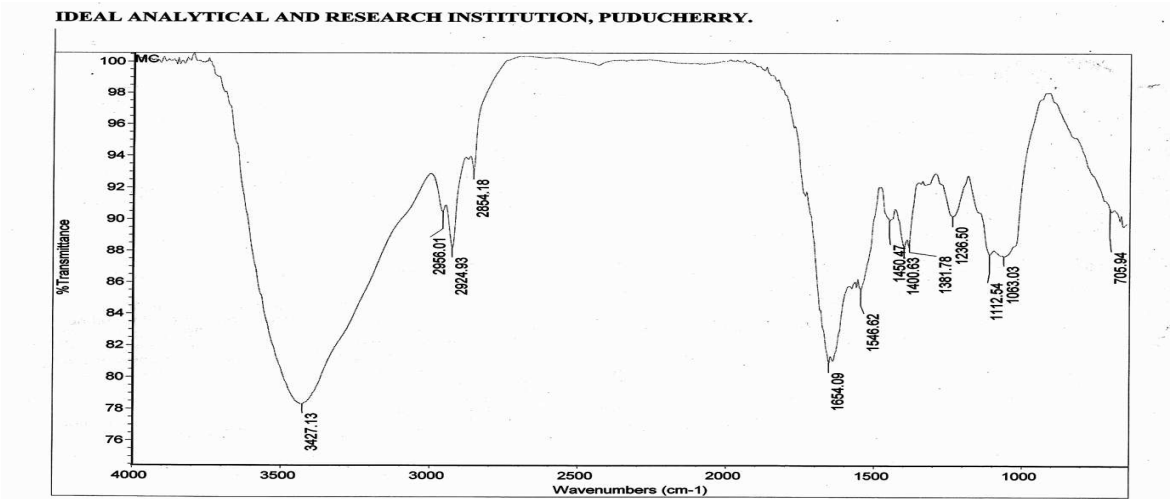


Fig.4 FTIR spectra of *Pseudomonas* sp. *PRK786*: mercury sulphate treated surface

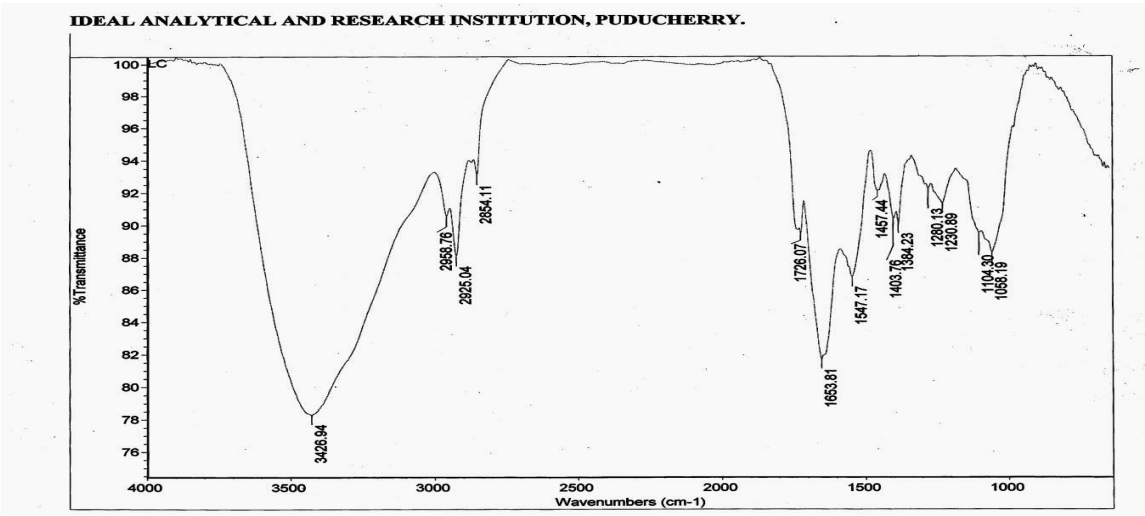


Fig. 5 FTIR spectra of *Pseudomonas* sp. *PRK786:* lead acetate treatedsurface

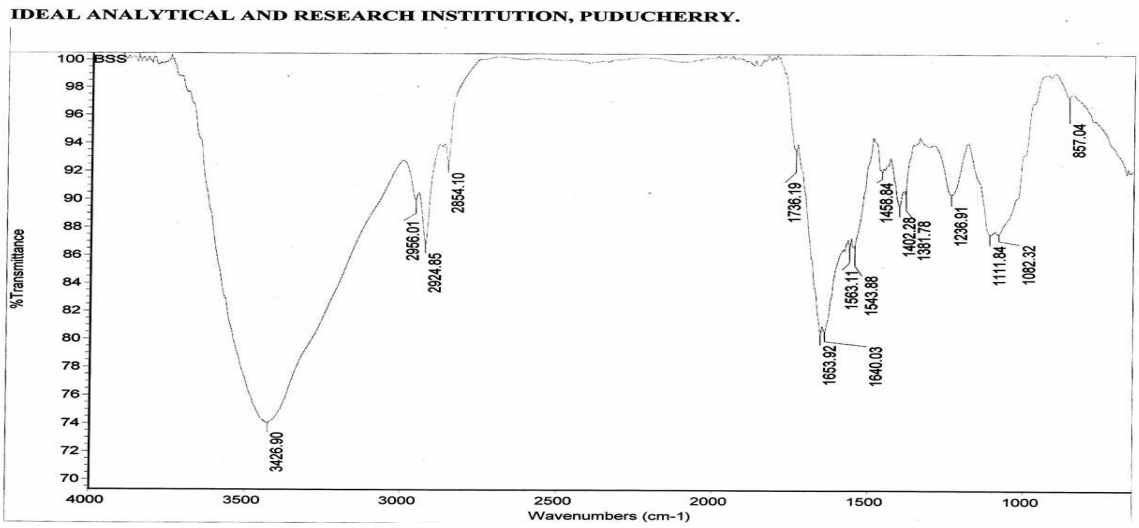


Fig.6 FTIR spectra of *Pseudomonas* sp. *PRK786* : bismuth sulphate treatedsurface

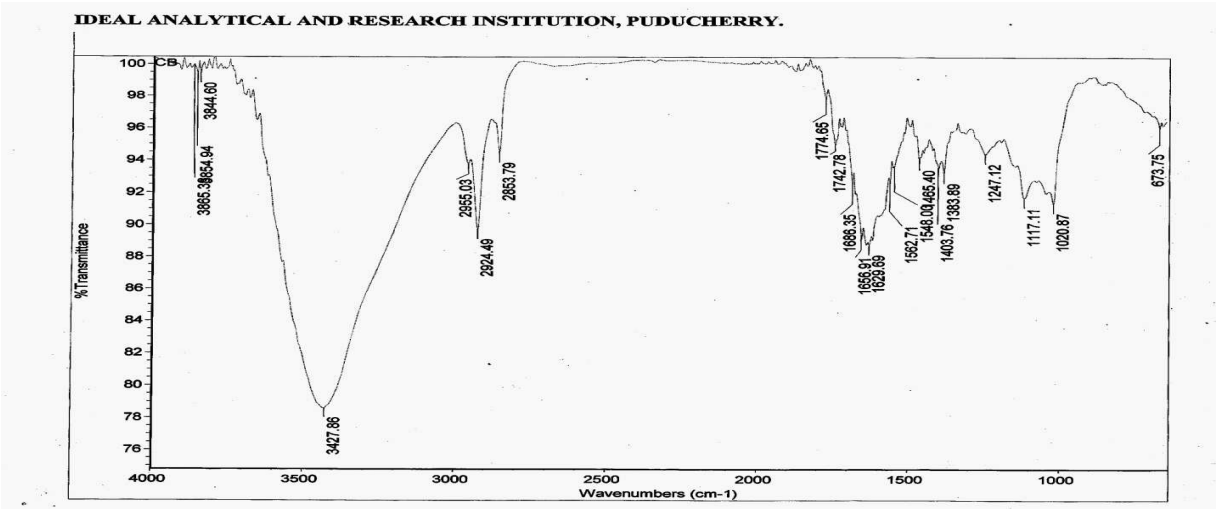


Fig.7 FTIR spectra of *Pseudomonas* sp.*PRK786* : Potassium dichromate treated surface

Band – I

The previous study also exhibit broad absorption band between 3500 and 3200 cm^{-1} due to bonded –OH stretching vibration and in this range of stretching vibration of amine group located at around 3415.42 cm^{-1} (Control), 3427.86 cm^{-1} (Chromium), 3426.94 cm^{-1} (Bismuth) and 3427.13 cm^{-1} (mercury) and 3426.94 (lead).

Band – II

The absorption peaks around at 2853 to 2873 cm^{-1} indicates CH_3 vibration, generally symmetric CH_3 vibration occur a 2885 to 2865 cm^{-1}. While CH_3 asymmetric stretching vibration occur at 2975 to 2950 cm^{-1}, above these peaks was not responsible for bioadsorption heavy metals on the bacterial surface.

Band – III

The bands at 2927.09 Cm^{-1} (control), 2924.93 Cm^{-1} (Mercury), 2925.04Cm^{-1} (lead), 2924.49Cm^{-1} (Chromium) and 2924.85 Cm^{-1} (bismuth) can be assigned to the –CH Stretching vibration (indicator of alkyl chains) the same results has been observed in the previous reports [21, 22, 23, 9]

Band – IV

The IR analysis of biosorbent specifically the 1650-1620 cm^{-1} band indicated the existence of the amide I band of amide bond in poly-N-acetyglucosamine and the protein peptide bond present in biomass considered to be due to combined effect of double bond stretching vibrations (mainly C=O) and hydrogen bonding. The typical amides I band, C==O stretching vibration, appears strongly at 1654.99 cm^{-1} was observed only on the control bacterial surface, but treated bacterial surface has less abortion (Bismuth – 1653.92 cm^{-1} , Mercury – 1654.09 cm^{-1} , lead – 1653.81 cm^{-1}, chromium -1658.91 cm^{-1}. which were indicate chromium ion are may be strongly binds to the amide groups. The same result observed in the previous report [24].

Band – V

CH_3 symmetric absorption can occur both treated and control (control - 2961.50cm^{-1}, Chromium - 2955.03cm^{-1}, Mercury – 2956.01 cm^{-1}, Bismuth -2956.01 cm^{-1} and Lead – 2958.76 cm^{-1}). CH_3 asymmetric deformation can be absorbed both control and treated bacterial surfaces. The band are(control -1440.97cm^{-1} Mercury – 1450.47cm^{-1}, Lead – 1457.44cm^{-1}, Chromium – 1465.40cm^{-1} and Bismuth - 1458.84cm^{-1}) absorbed and also generally, the formation asymmetric deformation absorption falls on 1440 – 1470 cm^{-1}, which was indicated $–CH_3$ of acetyl moiety, so they may can't

responsible for bio adsorption of Heavy metals on bacterial surface.[9]

Band – Vl

We are seen the absorption bands around 1400Cm^{-1} on the treated bacterial surfaces alone (mercury – 1400.63Cm^{-1}, lead – 1403.76Cm^{-1}, Chromium – 1403.76Cm^{-1}, Bismuth – 1402.28 Cm^{-1}) but it is not observed in control Bacterial surface. The absorption bands around1400cm^{-1} is due to the vibration of C=O of COO- carboxy late ions and O–H carboxylate ions. Which were indicating to us, carboxyl late ions are may be responsible for adsorption of these heavy metals. [22]

Band – Vll

The other typical amide bands (amide III) located in 1381.11 cm^{-1} to 1383.89 cm^{-1}were identified both control and treated bacterial surfaces.

Band – Vlll

The peaks at around 1544.20 cm^{-1}, known as amide II, is contributed to a motion combining both the –NH bending and the –CN stretching vibration of the group –C(=O)–NH– in its transform.[23]

CONCLUSION

Heavy metals response behaviors of indigenous *Pseudomonas* sp. PRK786 was exhibited various significant results such as growth and sulphur oxidations. The corrosive sublimate (mercuric chloride)and salt of Saturn (lead acetate): completely suppressed the all behaviors due to inhibition of including glucose metabolism and sulphur oxidations. Preliminary mercury and lead ions have affinity to binds the glucose oxidizing enzymes (Lack of carbon source). Bichromate of potash (Potassium dichromate): moderately inhibited the growth and sulphur oxidations and moderately water-soluble (bismuth sulphate): exhibited no significant inhibition of both growth and sulphur oxidations. The FTIR analysis of bioadsorption of heavy metals on the bacterial surfaces results suggested the chemical groups are may be responsible for adsorption of these heavy metals. In future, Pseudomonas sp. PRK786 contains enzymes may be used for made up portable biosensors.

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