Extracellular synthesis of Fe-NPs by bacteria/biosurfactant, kinetic parameter and reduction of Cr (VI) from pollutant samples

ABSTRACT

Hexavalent Cr is most toxic metal and hazardous compound worldwide. It is also considered to be cellular oxidizing pollutant causing damage, mutagenic and hematopoietic effects to living systems. Present work is focused on synthesis of zero valent iron nano particles (Fe-NPs) using *Bacillus subtilis* SHB 13, *Achromobacter xylosoxidans* SHB 204 and Surfactin and their application for Cr (VI) detoxification (100 ppm) in synthetic solutions and polluted sludge, sewage and soil samples about 90-100 % reduction observed with above particles. Qualitative analysis, structural characterization of Fe-NPs, adsorption, and recovery of Cr (VI) were carried out using XRD, SEM and TEM. This work explains adsorption isotherm of Cr with iron nano particles (Fe- NPs), was best fitted with Langmuir model and it followed first order rate kinetic reaction. Microcosm studies were carried out using Fe-NPs in sludge, sewage and tannery samples and Cr (VI) was completely (100 %) removed within 72 h. In another microcosm experiment, bacteria were able to reduce (60-70 %) Cr (VI) from soils with and without moisture and glucose. In green house experiments both the bacteria were able to reduce Cr (VI) (65-90%) effectively from both polluted and non-polluted soil and there was increase in root length and shoot length of plant *L. esculentum* was observed and maximum plant biomass was observed with plants inoculated with SHB 204 and SHB 13.

Keywords: *Bacillus subtilis*, *Achromobacter xylosoxidans*, Langmuir, microcosm, *L. esculentum*

List of Abbreviations

Cr	Chromium
Fe-NPs	Fe-Nps
XRD	X-ray Diffraction
SEM	Scanning electron microscope
TEM	Transmission electron microscope
L	Lycopersicon
SEM-EDS	Scanning electron microscope Energy dispersive spectrophotometer
AAS	Atomic absorption spectrophotometer
FeCl3	Ferric chloride
HNO3	Nitric acid
PPM	Parts per million

1. INTRODUCTION

Environmental pollution due to industrial revolution has become a major global concern, adversely affecting physical and biological components of earth/atmosphere [1]. As per World Health Organization, about a quarter of the diseases encountered by human beings today are due to prolonged exposure to environmental pollutants. Since the industrial revolution, metal ions entry into ecosystem has increased rapidly. Unlike organic pollutants these are not biodegradable and picomolar concentrations are threat to life and environment [2].

Chromium (Cr) has been widely used in industries including metallurgy, petroleum refining, electroplating, dye making and manufacture of stainless steel and refractory materials [3]. Hexavalent chromium is toxic at 0.05 ppm, carcinogenic and mutagenic to animals as well as humans and decreases plant growth [4,5]. Biological treatment of Cr by methods such as biosorption, bioaccumulation, biomineralization, bioleaching using microorganisms and their metabolites is efficient, less expensive and need of the day [6, 7].

In the current scenario there is fast growing interest in using nanoparticles (NPs) for heavy metal removal. Biosynthesis of nanoparticles by microorganism is a green and eco-friendly technology and use of these nanoparticles in medicine, agriculture, cosmetic industry, drug delivery, biochemical sensors and bioremediation is upcoming [8]. More studies to be carried out to improvise and standardize the method in not only eco-friendly synthesis but economical synthesis of these nano particles to be carried out. Iron nanoparticles (Fe-NPs) due to their super magnetic property and high adsorption ability can remove metals from

contaminated sites, sediments, sludge and sewage samples and can also be reused [9,10]. Surfactin is a cyclic lipopeptide biosurfactant produced by *Bacillus* sp. and is used as template and stabilizing agent for synthesis of silver and gold NPs [11]. In this study *Bacillus subtilis* SHB 13 was able to produce surfactin biosurfactant utilizing low-cost substrate (data published) and it was tested for its application in synthesis of Fe-NPs which in turn are used in bioremediation of Cr from polluted sites.

To discover the capability of biosorbents in reaching the absorption of Cr (VI), adsorption equilibrium and kinetic correlation was performed [12]. Most of the literature supports Langmuir and Freundlich models which are the best models for Cr (VI) adsorption on cell biomass and iron nano particles (Fe NPs) [13].

Few conventional water treatment methods are not always effective in removing contaminants such as metal ions. By products formed using these methods may cause to human health. Thus, Nanomaterials are used as alternatives in reduction of contaminants. These nanomaterials usage which are less than 100 nm size greatly differ from other methods in having electrical, mechanical, magnetic properties than other degradation methods. Nanomaterials are also are found to have high adsorbent property due to small size and large surface area and found to be relatively inexpensive. Various nanomaterials are extensively researched especially zerovalent Fe-Nps for their potential application in water and waste water treatment (14).

In heavy-metal polluted soils and marine samples, the new approach is the use of heavy metal-resistant bacterial strains capable of producing biosurfactants for increasing the metal removing efficiency. How ever the use of microbial surfactants has advantages over bacterial

strains as these surface-active compounds can directly chelate the metal ions from heavy metal contaminated soils. Lipopeptide such as surfactins produced by *Bacillus spp.* have potential activity for heavy metal, poly aromatic hydrocarbon and oil removal from contaminated sites (15), similarly our bacterial isolate *Bacillus subtilis* SHB 13 producing surfactin was able to reduce Cr (VI) to non-toxic form with improved activity in comparison and with greater stability.

Microbial synthesis of nano sized materials especially Fe-NPs can act as adsorbents and can be used in bioremediation. Fe-sulphide nano particles were reported to reduce Cr (VI) from simulated ground water (16) Surfactin is a cyclic lipopeptide biosurfactant produced by *pseudomonas* sp. and is used as template and stabilizing agent for synthesis of silver and gold NPs (17).

This study evaluates the use of Fe-NPs synthesized by *Bacillus subtilis* SHB 13, *Achromobacter xylosoxidans* SHB 204 and surfactin produced by *B. subtilis* SHB 13 for bioreduction of hexavalent chromium from polluted samples and further, kinetic studies were carried out. Fe-Nps (Fe-NPs), synthesized using supernatant of *B. subtilis* SHB 13 and *A. xylosoxidans* SHB 204 and surfactin and characterized using XRD, SEM-EDS and TEM. One gram of Fe- NPs synthesized by bacterial isolates showed complete bioreduction of chromium within 6 h of incubation and surfactin synthesized Fe –Nps took 7 h for complete reduction. Rate kinetics and adsorption isotherm studies showed that *B. subtilis* SHB 13 and *A. xylosoxidans* SHB 204 individually and in combinations, surfactin and Fe-NPs synthesized best fitted with first order kinetics and langmuer model as R^2 value was close to one. Microcosm experiments in sludge, sewage and tannery samples was carried out and it was observed that 80% of Cr (VI) reduction from polluted samples. Fe-NPs from SHB13,

SHB 204 (within 2 h) and surfactin (within 1 h) was also effective adsorbent in more than 90 % Cr (VI) reduction from these polluted samples. Microcosm experiments were also conducted for chromium reduction from polluted soil samples using bacteria in both moisture and glucose containing conditions and it was observed that more than 60 % reduction of Cr from the samples with improvement in root length and shoot length in comparison with plants without inoculation of isolated bacteria such as SHB 13 and SHB 204. Results indicate that Fe-NPs is more efficient at reducing Cr (VI) which can be attributed to the much higher specific surface area provided by Fe-NPs compared to other nanoparticles. These results demonstrate that very low concentrations of Fe-NPs can be used to reduce substantial amounts of Cr (VI) which is economical and cost efficient using bacterial isolates and surfactant which in turn showed greater stability.

2. MATERIALS AND METHODS

2.1 Cr (VI) solution preparation, biological synthesis of Fe-Nps (Fe-NPs) by *Bacillus subtilis* SHB 13, *Achromobacter xylosoxidans* SHB 204 and Surfactin

A stock solution of $K_2Cr_2O_7$ (1000 mg l^{-1}) (1000 ppm) in double distilled water was prepared, different metal concentrations were obtained by diluting from stock to get metal concentrations of 10-100 mg l^{-1} , 10-100 ppm.

Biosynthesis of Fe nano particles was done by using *B. subtilis* SHB 13, *A. xylosoxidans* SHB 204, 24h cell supernatant containing chromium reductase enzyme and surfactin produced by *B. subtilis* SHB 13 as reducing agents. Above bacteria were isolated from

polluted samples collected from industrial areas based on their tolerance to varied heavy metals such as chromium, nickel and arsenic at high concentrations in comparison to other isolates found in the polluted samples (Data published). To cell supernatant (100 ml) and 1% solution of surfactin 10 mM of filter sterilized $FeCl_3$ solution was added and incubated for 24 - 48 h, precipitate formed was centrifuged, washed with absolute ethanol and characterized using UV – visible spectrophotometer, X- ray diffraction (XRD), scanning electron microscopy – energy dispersive spectrophotometer (SEM-EDS) and transmission electron microscopy (TEM)

2.2 Cr (VI) reduction by synthesized Fe-NPs of bacteria and surfactin from Tris Buffer

Batch experiments were carried out to study ability of Fe NPs in adsorption of Cr (VI) from Tris buffer. Standardization studies were carried out with varied concentration of Fe-NPs ranging from 100 mg to 500 mg and it was observed that concentrations ranging from 250-500 mg 100 ml^{-1} was effective in Cr reduction. 10 ppm of Cr (VI) containing solutions was added to 250 ml conical flask with different concentrations (250 and 500 mg 100 ml^{-1}) of Fe-nano particles synthesized by culture supernatant of SHB 13, SHB 204 and surfactin separately. After the results were procured with above experiments, concentration of Fe – NPs were increased to 1000 mg 100 ml^{-1} and hexavalent chromium concentration were increased to 100 ppm as most of the contaminated samples consists of Cr (VI) > 10 ppm. Above flasks containing tris buffer with 100 ppm Cr and 1000 mg 100 ml^{-1} Fe-Nps of SHB 13, SHB 204 and surfactin were incubated separately for 1-8h at 37 $^{\circ}C$. After incubation at different time intervals samples were drawn and remaining Cr (VI) analyzed using atomic absorption spectrophotometer (AAS) (AAnalyst, 700, Perkin elmer USA to observe for reduction in Cr concentrations.

2.3 Adsorption isotherms and kinetics of adsorption using modeling studies of bacterial biomass/ surfactin/ Fe-NPs

Optimum biomass of each bacterial culture was dispersed in a desired concentration that ranged from 1 mg ml^{-1} to 10 mg ml^{-1} for Cr (VI) metal. Adsorption of Cr (VI) by synthesized Fe-NPs was assessed. Flasks were incubated for a period and at the end of which the residual concentrations were determined. Data obtained were presented using Origin pro software [18].

2.4 Microcosm studies for bioremediation based on bio-reduction of Cr (VI)

At Patancheru, industrial area was selected for collection of effluent sewage and sludge samples where wastes of industries are dumped. Another water sample from Katedan area was collected which had the dumping of textile processing, bleaching, dyeing, printing and tannery industry. Sludge, Sewage and tannery samples were collected in sterile bottles and processed within 6h of collection. Cr, Ni and Pb concentration was determined using AAS. Above processed sample was also sent for physical examination to Institute of preventive medicine water analysis center (Table 1). After processing of these samples 1000 mg synthesized Fe NPs were added to 250ml flasks containing 100 ml pollutant samples in triplicates and incubated at 37 °C at regular time intervals of 120 minutes, samples were drawn and observed for reduction in Cr concentration using AAS.

2.5 Influence of soil moisture and glucose amendment in polluted soil sample for bioreduction of chromium (VI)

In microcosm study, metal contaminated soils were collected from different paint impregnated plants from Jeedimetla. Varied treatments were carried out as explained below to determine in detail if the mobilization of chromium was enhanced as a result of addition of moisture (0, 50 and 100%) and glucose (1%) to soil. For this experiment, 30 g of soil samples with 100 ppm Cr (VI) was mixed in Petri dish and different amendments of moisture, glucose was made separately (Table). These samples were inoculated with 2 % actively growing culture of SHB 13 and SHB 204 and incubated at room temperature 30 days. Soil samples (one gram) were collected every 7^{th} day sample was dissolved in water, heated and the samples were processed by HNO_3 treatment and were filtered. These filtered samples were analyzed for Cr (VI) concentration using AAS.

2.6 Bioreduction of Cr from contaminated soils by bacteria and its effect on plant growth traits

In another microcosm experiment using tomato as host plant, green house study was performed to evaluate the effect of polluted soils (Jeedimetla) on plant growth parameters, chromium uptake in root, shoot and leaf samples, bioreduction of chromium in soil samples. For this experiment, pots were filled with 500 gm of soil maintained with 50% moisture. Pots of varied treatments containing seedlings of *L. esculentum* were kept at 26-28 ºC in a greenhouse; sterile distilled water was added to each pot every alternate day to maintain moisture level of 50%. To observe for plant growth traits, after 30 days, plants were removed from pots, and each plant was shaken carefully to remove the bulk soil. The soil still adhered to the roots was removed by washing with distilled water. Plant growth parameters, such as shoot, root length was taken and biomass (dry weight) was taken by drying until constant weight. To observe for Cr (VI) absorption by different parts of *L. esculentum* plants, samples

were harvested every 7th day and digested. From the inoculated pots soil samples were drawn, processed and observed for reduction of Cr (VI) till 30th day of incubation. Sampling was done thrice, 7th, 15th and 30th day for different studies as mentioned above. Treatments used were: T1 = Polluted soil without Cr amendment, T2 = Pollutant sample + Cr (VI), T3 = Polluted soil + Cr (VI) + SHB 13 (1gm), T4 = Pollutant sample + Cr (VI) + SHB 204 (1 gm).

Note

Based on statistical analysis ANOVA- two factor without replication with standard error bars and grades in the alphabetical order are provided inclusive of the priority factor/ treatment used.

3. RESULT

3.1 Biologically synthesized Fe-Nps and their characterization

Synthesis of iron nano particles was carried out by amending filtered 10 mM $FeCl_3$ into culture supernatant of *B. subtilis* SHB 13, *A. xylosoxidans* SHB 204 and surfactin separately. These synthesised Fe-NPs were characterized by UV – spectrophotometer and result showed maximum absorbance in the range of 216-275 nm, XRD pattern scan was carried out for 30 min over a 2Θ angle range of 10 to 80° angle (Fig. 1). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies have been conducted to obtain information about the size, structure, and morphology of Fe-NPs. The SEM –EDS study is a

promising tool for dimensional analysis of nanoparticles. Image of above synthesized nano particle is shown in Figure 2 and 3.

3.2 Use of Fe-NPs of (SHB 13, SHB 204 & surfactin) for chromium reduction from Tris buffer

Fe-NPs (250 mg 100 ml^{-1}) synthesized by *B. subtilis* SHB 13, *A. xylosoxidans* SHB 204 separately were able to remove Cr (VI) (10 ppm) completely within 4 h of incubation. Fe-NPs (250 mg100 ml^{-1}) synthesized by surfactin could remove 75 % Cr (VI) within 5 h of incubation. As the concentration of nanoparticles increased there was maximum reduction of Cr (VI). It was observed that bacterial synthesized Fe-NPs (1000 mg 100 ml^{-1}) removed Cr (VI) (100 ppm) completely within 6 h of incubation and surfactin-Fe-NPs used at 1000 mg 100 ml^{-1} took 7 h for chromium reduction. In this study one gram of bacterial synthesised Fe-Nps, were able to reduce > 80 mg of Cr (VI).

3.3 Adsorption isotherms and rate kinetics of adsorption using modeling studies such as Langmuir, first order and second order

The correlation coefficient $R^2 = 1$ for pseudo-first order and $R^2 = 0.98301$ for pseudo-second order and kinetic equation states that pseudo-first order best fitted with experimental values as it is close to 1. This indicates the applied model adequately describes experimental data of biosorption of Cr. For biosorption of chromium using Fe-NPs coefficient of determination R of the model was 0.93501. This indicate the applied model effectively describes experimental data of biosorption of Cr (Fig. 4,5,6)

3.4 Bio-reduction of Cr (VI) from sludge, sewage and tannery samples using Fe-Nps produced by bacteria and surfactin

Different microcosm experiments were carried out using sludge, sewage and tannery samples for bioreduction of hexavalent chromium. Cr (VI) reduction/reduction from polluted samples using synthesized Fe- NPs by bacteria SHB 13, SHB 204, surfactin was observed within 120 minutes of incubation at 37 °C. In case of Fe- NPs synthesized by *B. subtilis* SHB 13, it showed complete Cr (VI) reduction within 50 minutes of incubation from sludge, 70 minutes from sewage sample and 90 minutes from tannery samples indicating Fe-NPs from SHB 13 are effective adsorbents (Fig. 7a). Whereas Fe-NPs synthesized by *A. xylosoxidans* SHB 204 showed Cr (VI) reduction more than 95% within 90 min of incubation in case of sludge sample, 80% from tannery and 65% from sewage sample (Fig.7b). Whereas, surfactin synthesized Fe-NPs showed complete reduction of Cr (VI) within 60 minutes from sludge sample, 90-100% reduction within 70-80 minutes from sewage and tannery samples respectively (Fig.7c). Difference in reduction of chromium concentrations from sample to sample could be due to presence of other pollutants in samples.

3.5 Microcosm experiment for chromium reduction from soil samples amended with moisture and glucose

Cr (VI) reduction by bacteria (SHB 13 and SHB 204) from polluted soil sample with 0, 50 and 100 % moisture concentrations along with amendment of 1 % glucose were studied, varied treatments presented in Table 2. Maximum Cr (VI) (more than 50 %) reduction was observed with SHB 204 with 50-100% moisture either in the presence or absence of glucose. SHB 13 also showed 60-75% Cr (VI) reduction at 50-100% moisture content with or without

glucose amendment, indicating both the bacteria can reduce Cr in presence of low nutrient conditions too Table 3.

3.6 Bioremediation of Cr (VI) from polluted soils by *B. subtilis* SHB 13 and *A. xylosoxidans* SHB 204 and its effect on plant growth

3.6.1 Effect on root and shoot length/ root and shoot biomass

Green house study was conducted using tomato as host plant to see the effect of bioremediation of pollutant in the presence of bacterial isolates SHB 13 and SHB 204. Root length and shoot length of the *L. esculentum* with varied treated soil samples was studied. It was observed that soils inoculated with bacteria (SHB 13 and SHB 204) showed higher root and shoot length of plants than uninoculated and Cr (VI) amended plants.

Polluted soil without chromium amendment showed 23.6 cm root length, polluted soil with Cr (VI) amendment showed (12.6 cm) 53 % of decrease of root length indicates toxicity of Cr (VI) on plant growth. However, polluted soil with SHB 13 showed (22.6 cm) and with SHB 204 showed (29 cm), which was significant root length in plants cultivated in polluted soil and chromium amended soil. This explains, the inoculation of bacteria has helped the elongation of root and ultimately supported plant growth. In terms of shoot length also similar result were observed, whereas polluted sample amended with chromium showed decrease of 36% of shoot length when compared to pollutant sample amended with bacteria, this indicates ability of bacteria to reduce Cr (VI) toxicity, details of treatments carried out are mentioned in materials and methods (Fig. 8).

Effect of Cr (VI) on root and shoot biomass of *L. esculentum* was studied in different treatments, it was observed that root and shoot biomass of *B. subtilis* SHB 13 and *A.*

xylosoxidans SHB 204 treated plants was more than plants without bacterial treatment (SHB 13 and SHB 204) shown in Fig 9. In plants grown in polluted soil, plant growth was comparatively better may be due to presence of minerals. Root biomass of T1 was 12.8gm, for T2 it was 5.7 gm, for T3 it was 13.5 gm, for T4 it was 17.2 gm. Maximum values of plant biomass were observed with plants inoculated with SHB 204 followed by SHB 13. However, plant biomass was less in both chromium amended and unamended soil. In a report by Soni *et al.,* 2014 shoot and root biomass and root and shoot length were improved due to pretreatment of Cr (VI) contaminated soil samples and inoculations with bacterial cultures.

3.6.2 Effect of Cr (VI) reduction from Cr (VI) amended and unamended soils

Effect of Cr (VI) absorption by root, shoot and leaf of *L. esculentum* plants were studied for all the treatments used in this study. In polluted soil, treatment (T1) without chromium amendment and no bacterial inoculation, root sample showed 12 ppm uptake, shoot sample showed 3 ppm and leaf sample 0.8 ppm and there was no chromium content in the soil (100% reduction). In T2, root sample showed 11.3 ppm chromium, shoot showed 6.3 ppm and leaf showed 0.82 ppm and chromium remained in soil was around 74%. Whereas in treatments T3 and T4 in which bacterial isolates SHB 13 and SHB 204 were inoculated, chromium uptake in root was 12.6 and 16ppm, shoot was 8.3 and 27.89 ppm and leaf were 1.69 and 1.89 ppm, chromium remaining in soil was 37 and 34 ppm respectively (Table 3).

Complete reduction of Cr (VI) was observed in pots containing polluted soil sample (T1), as it contained less (16 mg kg^{-1}) concentration of Cr (VI). With T2 there was least reduction (25 %) of Cr (VI) as it was not added with any bacterial culture and native flora of effluent was unable to reduce high Cr (VI) concentrations. T3 and T4 showed 63 and 66 % Cr (VI)

reduction respectively in which *B. subtilis* SHB 13 and *A. xylosoxidans* SHB 204 was inoculated which might have contributed in Cr (VI) reduction.

4. DISCUSSION

Based on experimental observations of this study, synthesized Fe-NPs using supernatant of *A. xylosoxidans* SHB 204, *B. subtilis* SHB 13 and 1 % surfactin were characterized using XRD, SEM and TEM. SEM image of synthesized nano particle are crystal and dendrite shape, which are indicative for providing increased surface area for interaction with metal ions. TEM images of Fe NPs synthesized after 24 h of incubation were observed and this showed the quasi particles of 100 nm size and diffraction spots were also observed, which indicated crystalline nature of nano-particles (19). These synthesized nanoparticles could absorb high concentrations of Cr (VI) in less period of time from synthetic solutions as well as contaminated environmental samples. And adsorption process is best fitted into Langmuir model and it follows first order kinetics. Fe-NPs and bacterial cultures could also effectively reduce Cr (VI) contamination from polluted water and soil samples reducing the pollutant load. The isolated bacterial isolates and Fe- NPs can be further explored effectively as a bioremediation tool in industrial application as well as large scale sludge treatment plants.

Iron nanoparticles can be prepared using varied methods such as physical, chemical and biological methods. In comparison to physical and chemical methods biological

nanoparticles synthesis is cost effective and competent method for large scale production of nanoparticles which can be used for bioremediation (20).

Extracellular synthesis of nanoparticles is usually through production of reductase enzymes such as nitrate reductase etc which is secreted by the microbe for metal reduction and nanoparticle synthesis (21). In our study extracellular synthesized Fe-NPs through Cr reductase enzymes produced by SHB 13 and SHB 204 and surfactin were characterized by UV – spectrophotometer which showed maximum absorbance in the range of 216-275 nm, XRD pattern scan was carried out for 30 min over a 2Θ angle range of 10 to 80 $^{\circ}$C. The sharp peak appeared at 44° angle for both SHB 13 and SHB 204 and 30° angle for surfactin matched with reported values of zerovalent Fe-NPs (22). In their study they synthesized zerovalent iron nanoparticles using a wet chemical technique in comparison economically high with our biologically synthesized low cost substrates. SEM image of synthesized nanoparticles are crystal and dendrite shape, which are indicative for providing increased surface area for interaction with metal ion. TEM images of Fe-NPs synthesized after 24 h of incubation were observed and this showed the quasi particles of 100 nm size and diffraction spots were also observed, which indicated crystalline nature of nano-particles. TEM results also shows that enzymes present on cytoplasmic membrane and within the cytoplasm might have reduced the Fe ions that diffuse through the cell wall as similar observation was reported about iron oxide nanoparticles prepared using a modified sol-Gel [23].

In accordance with the application of synthesized nanoparticles in Cr (VI) reduction, results indicate that Fe-NPs are more efficient at reducing Cr (VI) which can be attributed to the much higher specific surface area provided by Fe-NPs compared to other nanoparticles.

In this study one gram of bacterial synthesised Fe-Nps, were able to reduce > 80 mg of Cr (VI) and in a similar report, adsorption of 20 mg L^{-1} of chromium was observed using one gram of magnetite iron nano particles [24]. These results also demonstrate that very low concentrations of Fe-NPs can be used to reduce substantial amounts of Cr (VI) which is economical and cost efficient.

Microcosm experiments using isolated bacterial cultures and Fe-NPs were carried out and in one of our previous study *A. xylosoxidans* SHB 204 and *B. subtilis* SHB 13 biomass and enzyme was able to remove 80-100 % of Cr (VI) from sludge, sewage and tannery samples [25]. There are very few studies on microbial synthesized Fe NPs in Cr (VI) reduction from pollutant water samples which is also a cost-effective method in bioremediation. According to a study by [26], iron oxide nanoparticles were able to remove 70 % of mercury ions from waste waters. In another study 4g of zerovalent Fe-Nps were able to reduce copper and nickel metal ion from industrial waste water [27]. Thus, current study could also give effective metal uptake result in comparison [28].

Soil samples amended with moisture and glucose was carried out and it was observed that Cr (VI) reduction by bacteria (SHB 13 and SHB 204) from polluted soil sample with100 % moisture concentrations along with amendment of 1 % glucose. Maximum Cr (VI) (more than 50 %) reduction was observed with SHB 204 and SHB 13 either in the presence or absence of glucose. SHB 13 also showed 60-75% Cr (VI) reduction at 50-100% moisture content with or without glucose amendment, indicating both the bacteria can reduce Cr in presence of low nutrient conditions too.

However, many of the previous reports explain importance of carbon source to be effective for chromium reduction in contaminated samples. For example, gluconate appeared to be the most effective carbon source for Cr (VI) reduction with the *Ochrobactrum intermedium* Rb-2 strain [29]. Glucose was found to enhance the Cr (VI) reduction in *Agrobacterium radiobacter*, *Bacillus cereus*, *Escherichia coli* ATCC33456 and *Pseudomonas fluorescens* LB300 [30]

In the microcosm plant study, inoculated bacterial strains, SHB 13 and SHB 204 were able to tolerate 100ppm of chromium and other pollutants present in sample used for plant growth study and also able to remove the chromium in presence of the native flora. Similar findings were also observed in a study where seed germination and growth of *Helianthus annus* and *Cicer arietinum*, were markedly affected by the presence of chromium salts [31] and [32]. Cr (VI) decrease in root and shoots length, but bacterial inoculation of soil significantly enhanced growth of seed, *Helianthus annus* when compared with uninoculated controls. According to a report by [33] strain of *Microbacterium* sp. SUCR140 reduced the chromate toxicity from artificially amended Cr (VI) containing soils resulted in improved growth and yields of plants compared to control. In a report by [34] *Bacillus and Arthrobacter* sp. were able to reduce Cr (VI) from contaminated soil and [32] also reported that the *Ochrobactrum intermedium* inoculation caused a decrease in chromium uptake into seedlings as compared to their respective non-inoculated control.

5. CONCLUSION

Based on experimental observations of this study, it shows that synthesized Fe-NPs using supernatant of *A. xylosoxidans* SHB 204, *B. subtilis* SHB 13 and 1 % surfactin can absorb

high concentrations of Cr (VI) in less period of time from synthetic solutions as well as contaminated environmental samples. Adsorption process is best fitted into Langmuir model and it follows first order kinetics. Fe-NPs and bacterial cultures could also effectively reduced Cr (VI) contamination from polluted water and soil samples reducing the pollutant load. The above bacteria and Fe- NPs can be further explored effectively as a bioremediation tool in industrial application as well as large scale sludge treatment plants.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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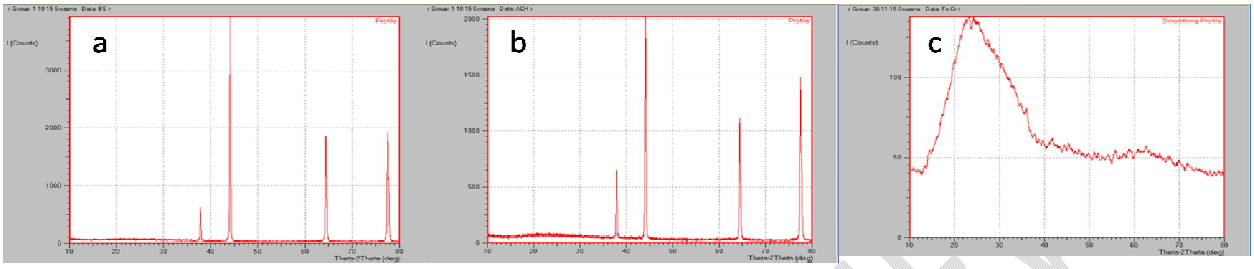

Fig.1. XRD pattern of Fe NP a. SHB 13 b. SHB 204 c. Surfactin

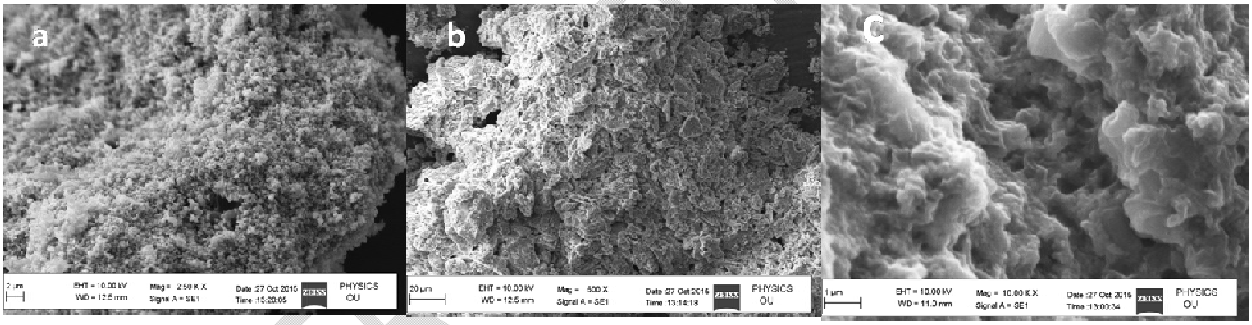

Fig. 2. SEM images of Fe NPs Synthesized by a. *B. subtilis* SHB 13 b. *A. xylosoxidans* SHB 204, c. 1% Surfactin

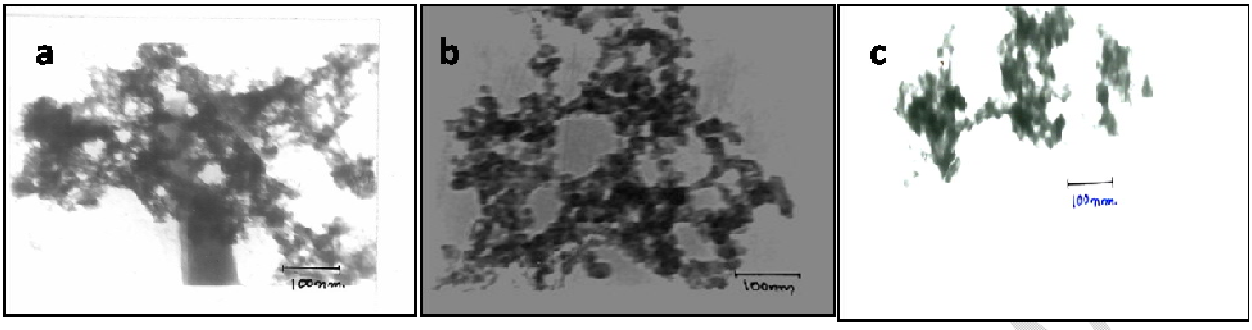

Fig. 3. Transmission electron microscopic image of Fe-NPs Synthesized by a. SHB 13 b. SHB 204 c. 1% surfactin

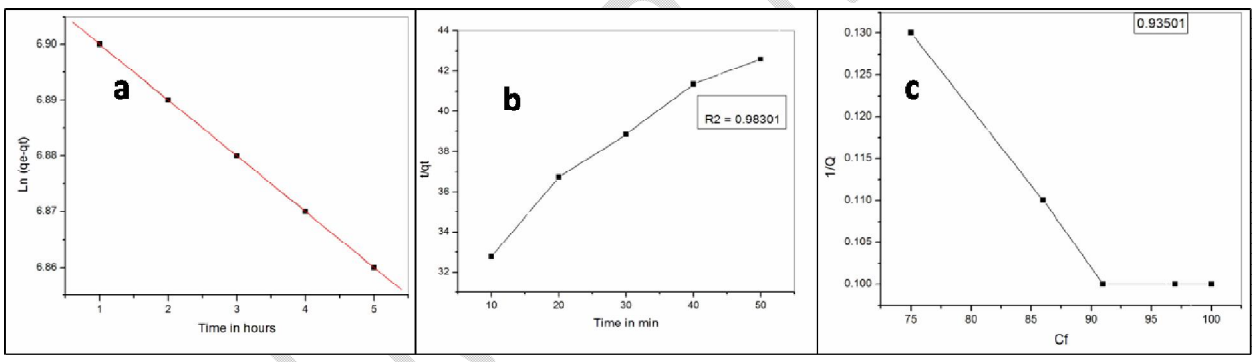

Fig.4. a. First order b. second order kinetics c. Langmuir model of Fe-NPs synthesized by A. xylosoxidans SHB 13

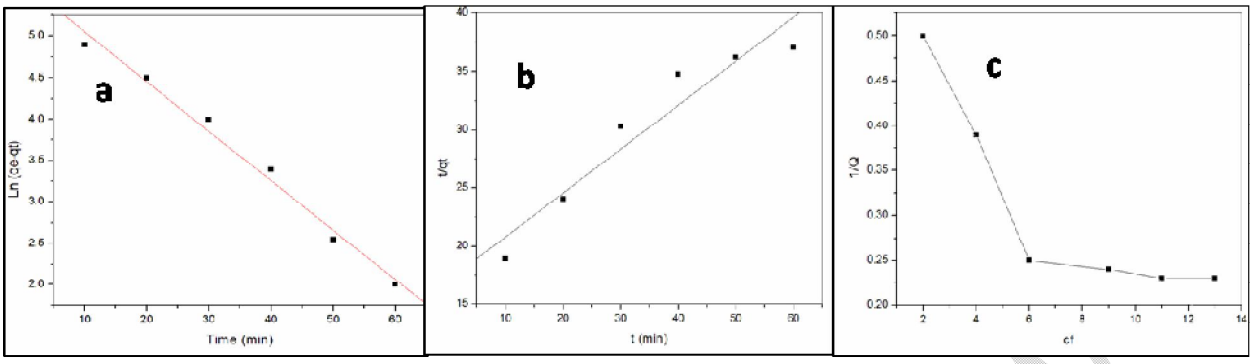

Fig. 5. a. First order b. second order kinetics c. Langmuir model of Fe-NPs synthesized by B. subtilis SHB 204

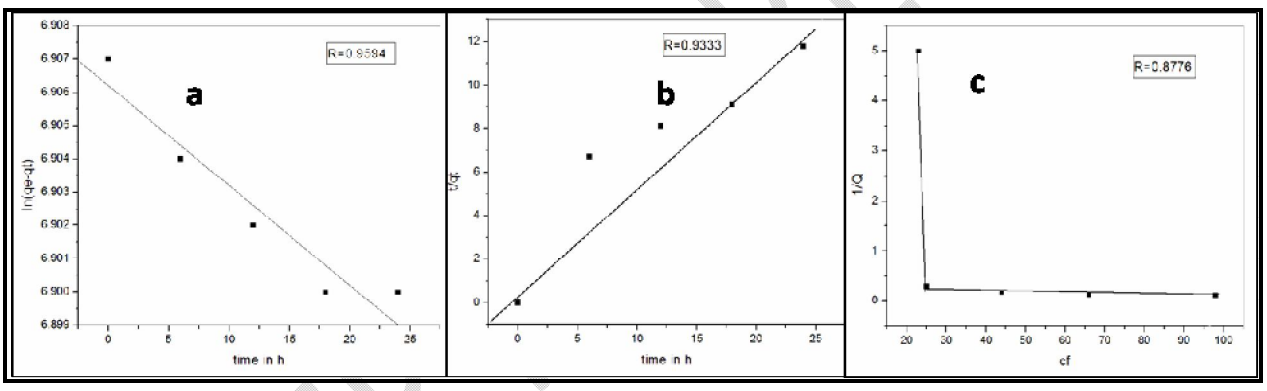

Fig.6. a. First order b. second order kinetics c. Langmuir model of Fe-NPs synthesized by surfactin synthesized by B. subtilis SHB 13

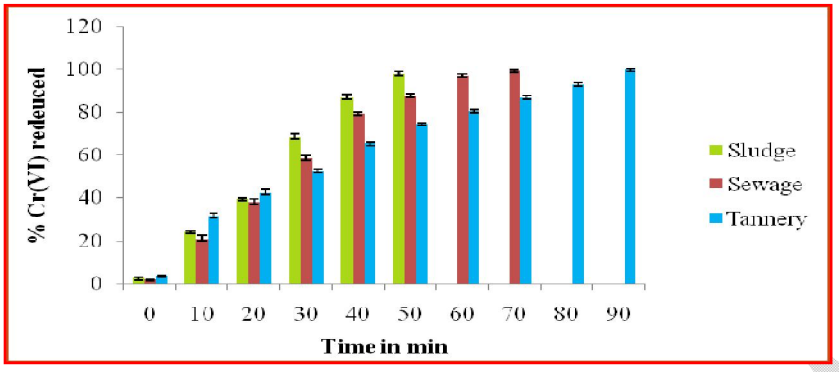

Fig.7a

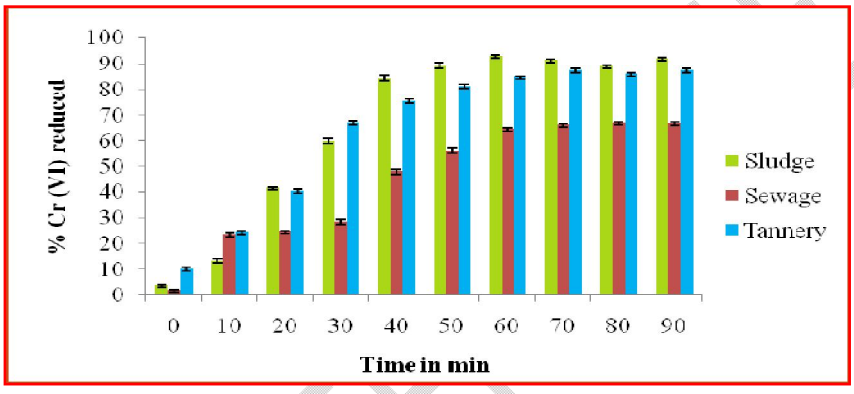

Fig.7b

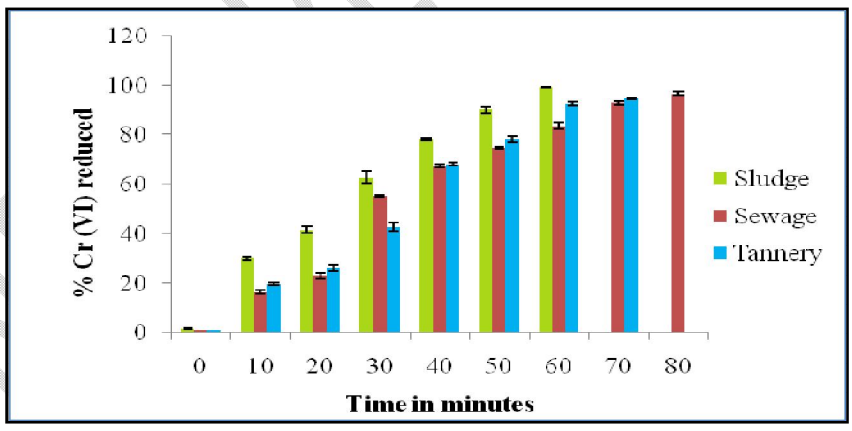

Fig. 7c

Fig.7. Percentage Cr (VI) reduction from polluted sample by Fe-NPs synthesized by (a) SHB 13, (b) SHB 204, (c) Surfactin

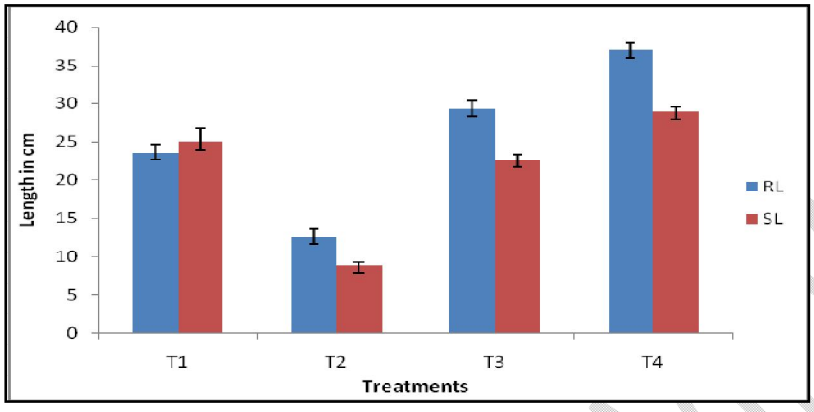

Fig.8. Effect of Cr (VI) on root length and shoot length of *L. esculentum* plants

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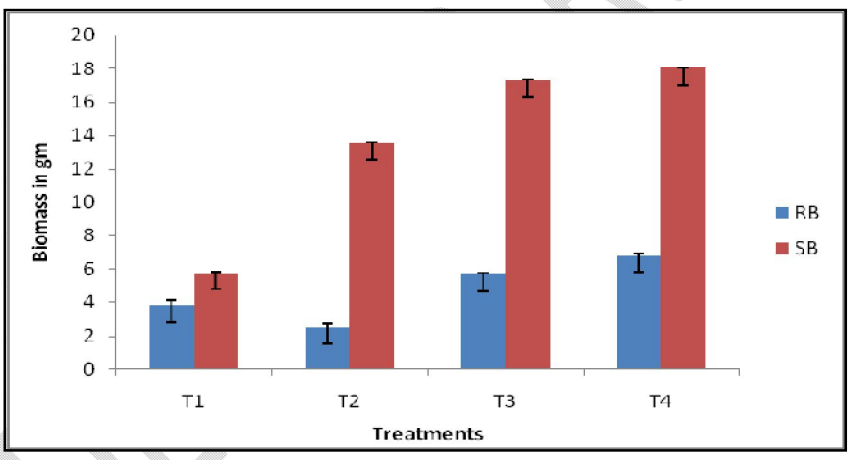

Fig.9. Effect of Cr (VI) on root biomass and shoot biomass of *L. esculentum* plants

Table1 Physiochemical characteristics and concentration of metal ions present in Industrial samples collected from water, sewage and sludge

Character studied	Type of sample		
	Sewage	Sludge	Tannery
Color (Hazen units)	30	40	30
Turbidity (Nephlometric turbidity unit)	4.6	7.0	2.3
Odour	U.O	U.O	U.O
pH	7.5	7.6	2.3
Electrical conductance at 25 $^{\circ}$C (Micro mohs cm^{-1})	2000	2204	2028
Total dissolved solids	1320	1454	1338
Total alkalinity	315	385	415
Ammonical Nitrogen (mg l^{-1})	Nil	Nil	Traces
Nitrite (mg l^{-1})	Nil	Nil	0.07
Sulfate (mg l^{-1})	135	160	108
Chloride (mg l^{-1})	310	370	345
Fluoride (mg l^{-1})	1.17	1.29	1.25
Chromium (mg l^{-1})	16	23	98
Nickel (mg l^{-1})	22	18	35
Lead (mg l^{-1})	24	14	56

Table 2. Soil microcosm treatments at varied moisture concentrations and glucose Amendments

Treatment	% moisture	Bacterium	Amendment
T1	0	*B. subtilis* SHB 13	-
T2	0	*B. subtilis* SHB 13	1% glucose
T3	0	*A. xylosoxidans* SHB 204	-
T4	0	*A. xylosoxidans* SHB 204	1% glucose
T5	50	*B. subtilis* SHB 13	-
T6	50	*B. subtilis* SHB 13	1% glucose
T7	50	*A. xylosoxidans* SHB 204	-
T8	50	*A. xylosoxidans* SHB 204	1% glucose
T9	100	*B. subtilis* SHB 13	-
T10	100	*B. subtilis* SHB 13	1% glucose
T11	100	*A. xylosoxidans* SHB 204	-
T12	100	*A. xylosoxidans* SHB 204	1% glucose
Control 1	No moisture	*B. subtilis* SHB 13	-
Control 2	No moisture	*A. xylosoxidans* SHB 204	-

Table 3. Cr (VI) reduction by SHB 13 and SHB 204 from polluted soil samples amended with moisture and glucose

Cr (VI) reduction from soil sample (%)			
Days / Treatment	7th	15th	30th
T1	6.3±0.33	16±0.33	45±0.33
T2	8±0.01	42±0.1	62±0.22
T3	14±0.67	37±0.21	70±0.33
T4	22± 0.33	46±0.33	73±0.10
T5	18±0.1	32±0.67	74±0.31
T6	23±0.6	32±0.66	74±0.66
T7	25±0.33	48±0.33	75±0.1
T8	32.6±0.7	59±0.66	75±0.67
T9	22±0.1	53±0.1	60±0.67
T10	29±0.1	60±0.67	71±0.1
T11	34±0.7	53±0.1	53±0.67
T12	35±0.33	65±0.67	64±0.66
Control 1	0.65±0.53	0.01±0.33	0±0

Table 4 Cr (VI) reduced by soil sample and absorption by root, shoot and leaf of plant (l. esculentum) at 30 th day

Treatment	**Soil**	**Root**	**Shoot**	**Leaf**
T1	100 ±0.22	12±0.13	3±0.54	0.8±0.11
T2	23±0.29	11.33±0.69	6.33±44	0.82±0.19
T3	63.33±0.90	12.66±0.68	8.33±0.12	1.69±0.32
T4	66.33±0.88	16±0.11	27.89±0.61	1.89±0.34