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Title - Extraction of Mg from hydrodesulfurization catalyst waste via *Pseudomonas luteola*

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Abstract

Catalysts play an indispensable role in the production of fuels in the petroleum refining industry. The rapid development of petroleum industry has led to increasing demand for catalysts. Petroleum refining involves the conversion of crude oil into a wide range of valuable products, with fuels being among the most important ones, including gasoline, diesel, jet fuel, and others Petroleum refineries generate and dispose of a large amount of solid waste. This waste contains toxic and hazardous components such as Mg, Mn, Cd, Cr, Fe, Pb, Al, V, Mo, Ni, As, and Fe. Apart from the environmental benefits, metal recovery from spent hydrotreating catalysts also holds significant economic advantages. Valuable metals like molybdenum, nickel, and magnesium have substantial market value, and their recovery can offset the costs associated with the disposal of spent catalysts. Moreover, recycling metals from spent catalysts can provide a more cost-effective source of these metals compared to traditional mining and refining methods. Heavy metals exert toxic effect on human beings. They accumulate and thereby disrupt function in vital organs such as the heart, brain, kidneys, bone, liver, and glands. Many techniques are reported for detoxification and extraction but they are very expensive and their secondary waste is also toxic. So, due to less energy consumption and minimum secondary waste generation, microbial treatment utilizing biosurfactant can be a promising eco-friendly, efficient, and cost-effective approach for various applications, particularly in environmental remediation and industrial processes.

Keywords: Petroleum refinery, Hazardous components, Detoxification, Microbial treatment Biosurfactant

1. Introduction

The increasing world population and enormous economic development resulted in rapid growth in many fields such as agriculture and industry, and the environment is becoming more polluted. During refining process in petroleum industry, various catalysts are used *viz.* HDS, FCC and HPC. Of these, HDS catalyst plays an important role in separation of sulphur and natural gas from crude oil during the petroleum refining. Rapid demand of hydrocarbons in last decades, resulted in more and more oil extraction and refining. The role of the catalysts is indispensable for the production of fuels in the petroleum refining industry. The life cycle of the catalyst will end when its activity drops below acceptable limits and are known as spent catalyst and are a nuisance to the environment if not treated before dispensing them. So, once the catalysts are deactivated, they are then either regenerated or processed to recover valuable components [1]. It has been reported that the amount of spent catalysts is estimated to be in the range of 150000–170000 tons per year [2]. This indicates that the processing of heavy oils in the petroleum refining industries is growing. The oil refining process is a complex of many processes by which the crude oil is converted to several more useful products such as gasoline, kerosene, naphtha, diesel, fuel oils, asphalt, and liquefied petroleum products. The crude oil by itself is generally not useful. Its products can be used as transportation fuels, heating fuels, lubricants, or as feeds for other petrochemical products such as plastics, polyesters, nylon, and solvents. Those valuable products are the results of tens of refining processes through which the crude oil is treated. Catalysts are essential for the production of gasoline and diesel fuel, jet fuels, heavy oil hydrocarbon, and petrochemical in the petroleum refinery industry. Among the several catalytic hydrotreating operations, a hydro-desulfurization (HDS) process is one of the major processes for converting crude oil into several clean fuels and other petroleum products [3]. The importance of catalysts to chemical processes is enormous. HDS activity is influenced by many factors such as operating conditions (e.g., temperature, pressure, weight hour space velocity (WHSV), and catalyst average particle diameter), catalyst type, and reactor type (e.g., fixed bed, moving bed, slurry, and) [4]. Two kinetic models are often utilized in studying an HDS process, the power-law model (PLM) and the Langmuir–Hinshelwood model (LHM). PLM is utilized to depict the global rate of reaction, while LHM is utilized to

take into consideration the resistances of species transfer through reactions. Another efficient but rarely used model is the multi-parameter kinetic model [5]. One of the major problems related to the use of the catalysts in fuel refining is loss of catalytic activity with time due to loss of surface area, sintering, and/or deposition of various compounds of heavy metals like Magnesium (Mg) and Manganese (Mn) , Cadmium (Cd), Chromium (Cr), Arsenic (As), Lead (Pb), Vanadium (V) Molybdenum (Mo), Nickel (Ni), Iron (Fe) etc [6]. Natural heavy metals are metals with a high atomic weight and a density greater than 5gm/cm^3[7]. Compared with their physical properties and chemical characteristics, heavy metals are the most practical aspect. The changes provoked by the deactivation of the catalyst play a significant role in a large number of industrial processes. Spent HDS catalysts are considered waste and also the replacement cost with fresh catalysts is quite high. Spent catalysts have been classified as hazardous waste by the United State Environmental Protection Agency (USEPA). After a certain period of catalytic processing, the catalysts become contaminated with impurities. By landfill disposal or land accumulation, the deactivated spent catalyst may pose potential environmental problems affecting soil, plant, water, animals, and humans. The recycling and utilization of spent hydrotreating catalysts are important for the protection of the environment and the recovery of valuable metals. Therefore, it is extremely important to develop a process for the recovery of valuable metals from the spent hydrotreating catalyst in environmental and economic aspects. Magnesium has three stable isotopes, Mg^{24}, Mg^{25}, and Mg^{26}. The most common isotope is Mg^{24}, which is 79% of all Mg found on Earth. Mg^{25} and Mg^{26} are used to study the absorption and metabolism of magnesium in the human body. They are also used to study heart disease. Magnesium not only has stable isotopes but also has radioactive isotopes, which are isotopes that have an unstable nucleus. These isotopes are Mg^{22}, Mg^{23}, Mg^{27}, Mg^{28}, and Mg^{29}. Mg^{28} was commonly used in nuclear sites for scientific experiments from the 1950 to the 1970 [8].While the economic importance of magnesium has increased considerably in recent years. Access to magnesium is a growing worldwide concern in some industrial sectors. With the increasing use of low GHG emitting technologies, in particular, to combat climate change and transition to renewable energy, magnesium is one of the most critical and strategic materials. It's also essential to the aluminum market for its role in alloy and iron and steel sector cannot be overlooked either as they require magnesium for desulphurization. Moreover, transportation industries are increasing in parallel with the demand for lighter, fuel-efficient vehicles that reduce greenhouse gas emissions. Magnesium is a naturally occurring element and an essential nutrient for humans, animals, and plants. It is geologically ubiquitous. Its compounds are widely used in construction and medicine, and magnesium is one of the elements essential to all cellular life. Magnesium is mainly present as Magnesium toxicity causes diseases like muscle slackening, nerve problems, depression, personality changes, etc [9]. The disposal and storage of the spent catalyst are also subject to many stringent regulations. Generally, the spent catalysts are managed by three main methods such as, chemical or microbial treatment for the recovery of valuable metals, regeneration, reuse, and landfilling [10]. Spent catalysts are rich in metals values like manganese and magnesium, nickel, cadmium, etc. Much research has been done on metal recovery from spent catalysts by employing hydrometallurgy, pyrometallurgy, or the combination of the two routes [11]. Most of the research indicated that hydrometallurgical processes are preferred over pyrometallurgical processes in recovering valuable metals from spent catalysts due to low energy consumption, less emission of toxic gas, and secondary waste generation [12]. The recovery of metals from secondary waste is not possible due to the leaching agents used in a hydrometallurgical method that are hazardous to the environment. Pyrometallurgy employs the thermal treatment to bring about physical and chemical transformation in the materials to enable recovery of valuable metals. So, the third and alternate process known as bioleaching is proposed in the present study for extraction of metals in an eco-friendly manner. Bio leaching process utilizes microorganisms like bacteria or fungi or their metabolites for extraction of metals. So, nowadays cleaning and extraction through biosurfactant producing bacteria is in high demand as it is a bioactive compound that is produced by microorganisms and is eco-friendly and non-hazardous surface active agent. Use of biosurfactant is becoming cost-effective technology as it also be produced from agro-industrial waste. The characteristics and advantages have drawn significant interest in exploring biosurfactants' potential applications in various fields, including bioremediation, enhanced oil recovery, food processing, cosmetics, and pharmaceuticals, among others. However, it's important to note that the effectiveness and suitability of a specific biosurfactant for a particular application may depend on its chemical structure and the specific requirements of the process. Biosurfactant possess a strong affinity for heavy metals, resulting in the formation of a biosurfactant–metal complex. They have unique property Ongoing research in this area aims to extract magnesium (Mg) through biosurfactant producing bacteria.

2. Material and Method

2.1 Sample collection and Preparation for Analysis

The solid HDS spent Catalyst waste was obtained from a site near Mathura Refinery situated in Mathura, Uttar Pradesh, India. Preliminary, the received catalyst from the refinery was first washed with acetone to remove soluble organic matter and then

dried overnight in an oven at 80°C. The shape of the catalyst was cylindrical and it was ground to desired particle size by using percolate mesh/or wire dia. The obtained powder material was mixed thoroughly to get a homogenized sample for chemical characterization and subsequent leaching experiments. The chemical composition of the spent HDS catalyst was determined by the dissolution of the catalysts in concentrated hydrofluoric acid. The concentration of metals in the resulting solution was measured by Inductive Coupled plasma optical emission spectroscopy (ICP-MS) [13].

2.2 Minimum inhibitory concentration (MIC) of Mg^{+} at different concentrations

The selected bacterial strains was also tested for their Mg tolerance to different Mg concentrations in increasing order such as 50, 100, 200, 400, 600, 800, 1000, 1200, 1400 mg/L by spreading them on the nutrient agar plate containing the respective Mg concentration. MIC was determined for each strain by visual growth monitoring to perform an Mg biosorption study from the aqueous system. For the biosorption study, the bacterial biomass was prepared by first growing the strains in the nutrient broth (NB) medium for 72 h at 37±2°C then the grown culture media were autoclaved for 20 min followed by centrifugation and washing the biomass thrice with 1% sterile saline water (NaCl), then dried in an oven at 60 °C and ground to a fine powder using a mortar pestle [14]. The batch biosorption study was conducted using 250 mL Erlenmeyer flasks containing 100 mL of NB media having different initial Mg ion concentrations of (10, 50, 100, 150, and 200 mg/L) with 0.1 g of bacterial biomass for 120 min, at 150 rpm shaking speed and pH 6.0[14].

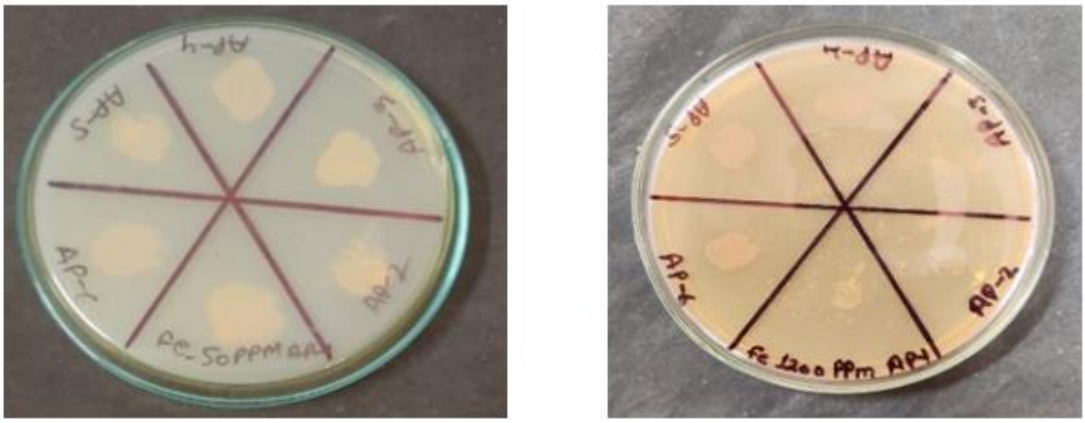

Fig-1: MIC of Mg^{+} at differernt concentration.

Mg+ (ppm)	AP-1	AP-2	AP-3	AP-4	AP-5	AP-6
50	+++	+++	+++	+++	+++	+++
100	+++	+++	+++	+++	+++	+++
150	+++	+++	+++	+++	+++	+++
200	+++	+++	+++	+++	+++	++
300	+++	+++	+++	+++	+++	+++
400	+++	+++	+++	+++	+++	++
500	+++	+++	+++	+	++	++
600	++	+++	+	++	++	++
700	+	++	+	++	++	+
800	+	++	+	+	+	-
1200	+	+	-	-	+	+
1400	-	-	-	-	-	-

+ (positive), -(negative)

Tabe-1: MIC of different bacterial isolates

2.3 Isolation and screening of potential bacterial strain for the extraction of Mg^{+}

The surface soil samples were collected from Mathura 27.3804° N, 77.6893° E, Uttar Pradesh, India. Soil samples were used to isolate bacterial strains that can be potentially used in extraction of valuable metals like Mg following the enrichment/acclimatization method. Briefly, during the enrichment process, 1 g soil was inoculated in a 250 ml Erlenmeyer flask containing 100 ml Mineral Salt Medium (MSM) in g/L of distilled water: 2.5g/L of $NaNO_3$, 3.0 g/L of KH_2PO_4, 7.0 g/L of K_2HPO_4, 0.01 g/L of $CaCl_2$, 0.5 g/L of $MgSO_4.7H_2O$ and trace element solution containing 0.116 g/L of FeSO4.$7H_2O$, 0.232 g/L of H_3BO_3, 0.41 g/L of $CoCl_2.6H_2O$, 0.008 g/L of $CuSO_4.5H_2O$, 0.008 g/L of $MnSO_4.H_2O$, 0.022 g/L of $[NH_4]$ $6MoO_{24}$, 0.174 g/L of $ZnSO_4$.and 2% oil as a sole source of the carbon. The pH of the medium was adjusted to 6.8. The flasks were incubated at 37°C on a rotary shaker at 200 rpm for 72 hrs. Continuous subculturing and shaking was carried out five times for enrichment. Isolation was carried out by inoculation on nutrient agar and morphologically distinct colonies were isolated and stored as a stock culture at 40 °C [15].

2.4 Screening of the selected isolate for biosurfactant production

Biosurfactant produced by the bacterial strains was screened by different methods. Drop collapse test was performed in the 96 microwell polystyrene plates (45). Thin coats of 10 W-40 oil was applied to each well and were equilibrated at 23°C for 24 h. Five microlitre of supernatant aliquot was deported into the centre of each well. Beaded drop indicates negative results while spread drop which collapse indicates positive results for the presence of biosurfactant[16]. Oil displacement test was determined by following the method of Ohno et al (1993). Briefly, 30 µl of engine oil was dropped on to the surface of 20 ml of distilled water in a 10 cm diameter petri dish till covering the total surface area of the water. Thereafter, 30 µL of isolated crude biosurfactant was added on to the surface of the engine oil layer in the Petridis. The diameter of the clear zones was measured and calculated [17]. In foam test bacterial isolate showing foaming in the medium as compared to negative controls were observed for reflected light which scattered like rainbow colour. Contents of the test tubes of minimal salt medium with the test strains (bacterial strains) showing foaming were subjected to centrifugation at 10,000 rpm for 20 min (4°C) [18]. Phenol Sulphuric acid test was used to detect the screening of type of biosurfactant glycolipids etc. Glycolipids production was detected according to the method of Dubois et al. (1956). Briefly, 1 ml of phenol was added to the little amount of crude biosurfactant. To the above mixture, 4 to 5 ml of concentrated H_2SO_4 was added drop by drop. Colour change was observed; development of yellow to orange colour indicated the presence of glycolipids [19]. CTAB test were used for screening for rhamnolipid production, the bacterial strain was spot inoculated on the CTAB plates (g/l Cetyl trimethyl ammonium bromide-0.2g; Methylene Blue-0.005g; Peptone-1.5, $MgSO_4$ -0.5, K_2HPO_4-1.0, $FeCl_3$ -0.1, KH_2PO_4-1.0, $CaCl_2$ -0.01,$MnSO_4$ -0.005, Agar 15 and Glycerol- 15ml). Thereafter, the plates were incubated at 30 °C for 7 days [20].

2.5 Molecular characterization of selected isolate

For molecular characterization extracted bacteria DNA. Further extracted DNA band was examined on 0.8% agarose gel having 1 µg/mL ethidium bromide under an ultraviolet (UV) transilluminator. The 16S ribosomal RNA gene was amplified using 5 µL of genomic DNA (as template DNA) and universal eubacterial primers (27F) 5-AGAGTTTGATCMTGGCTCAG-3 and (1492R) 5-CGGTTACCTTGTTACGACTT-3. The reaction mixture contains, template DNA (1 µL), 16S forward and reverse primer (400 ng), 16s Reverse, deoxynucleoside triphosphate (dNTP) 4 µL, 10X Taq DNA polymerase assay buffer 10 µL, Taq DNA polymerase enzyme (3 U/µL) 1 µL and Water X µL (Biokart, Pvt. Ltd. Bengaluru, Karnataka, India) in final reaction volume 100 µL. 16S rRNA gene fragments were amplified in an Optical 96-well reaction plate (Applied Biosystem Micro Amp) by running polymerase chain reaction (PCR) 35 cycle at initial denaturation, subsequent denaturation, annealing, extension, final extension on 95^0C, 94^0C, 50^0C, 72^0C and 72^0C temperature for 5min, 30s, 30s, 1.30 min and 1 min, respectively. Further, PCR products were resolved on 1% agarose gel and purified using a gel extraction kit and the purified PCR product was sequenced and subjected to basic local alignment search tool (BLAST) analysis using the online option available at http://www.ncbi.nlm.nih [22].

3. Results and discussion

3.1 Screening of isolates for Biosurfactant production

From the soil sample collected from the contaminated site a total of six morphological different isolates were (AP1, AP2, AP3, AP4, AP5, AP6,) obtained and screened for biosurfactant production. Only AP2 showed positive results for oil displacement, foam test, drop collapse test, phenol sulphuric acid test, CTAB test.

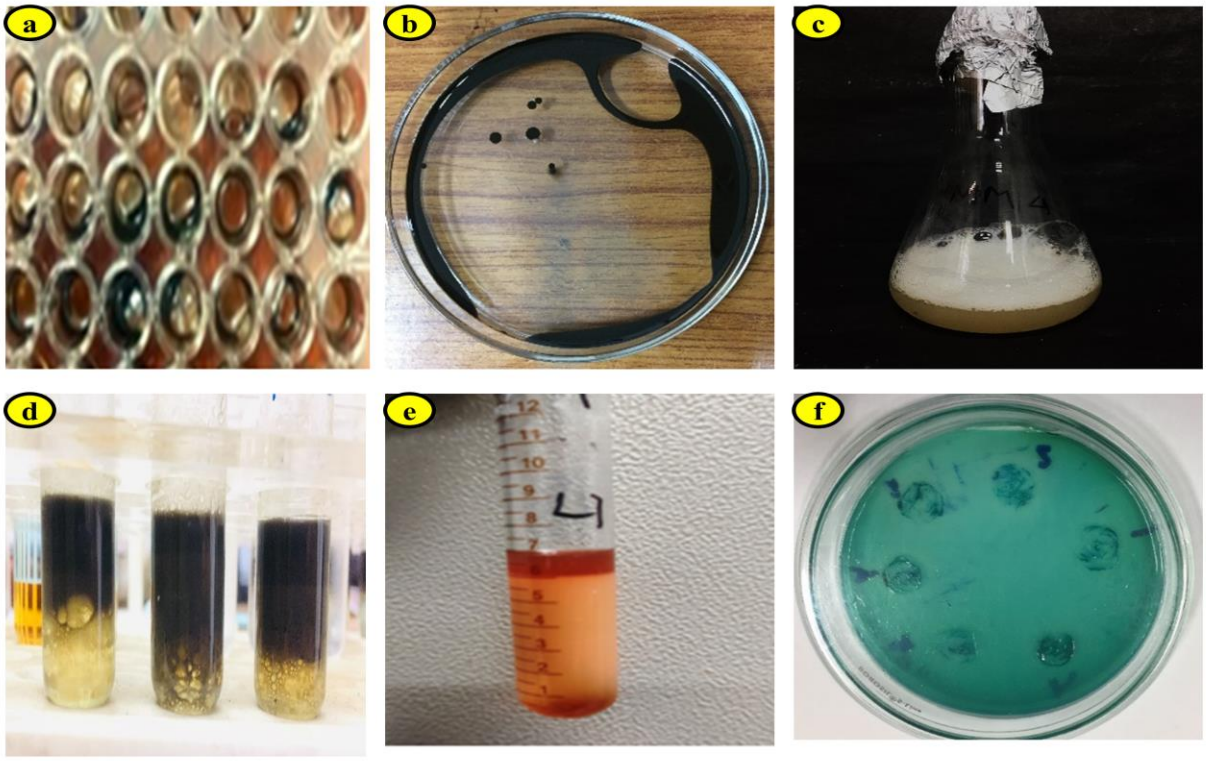

Fig-2: Drop collapse test (a), Oil displacement test (b), foam test (c), emulsification test (d), Phenol sulfuric acid test (e), CTAB test (f).

3.2 Characterization and identification of strain

AP2 (*Pseudomonas luteola*) is aerobic, non-spore-forming, gram-negative bacilli, and it is motile due to the presence of one or more polar flagella. Further biochemical tests such as glucose and citrate utilization were positive while indole, methyl red, Voges Proskaur's, citrate, glucose, adonitol, arabinose, lactose, sorbitol, mannitol, rhamnose and sucrose utilization were negative. Further, the PCR amplified 1500 bp long 16S rRNA gene sequences obtained from AP2 show closest relatedness (100%) with that of P. *luteola* strain. The phylogenetic tree is created by using Weighbor with alphabet size 4 and length size 1000. Sequence report confirmed that the isolated bacteria strain was P. *luteola*.

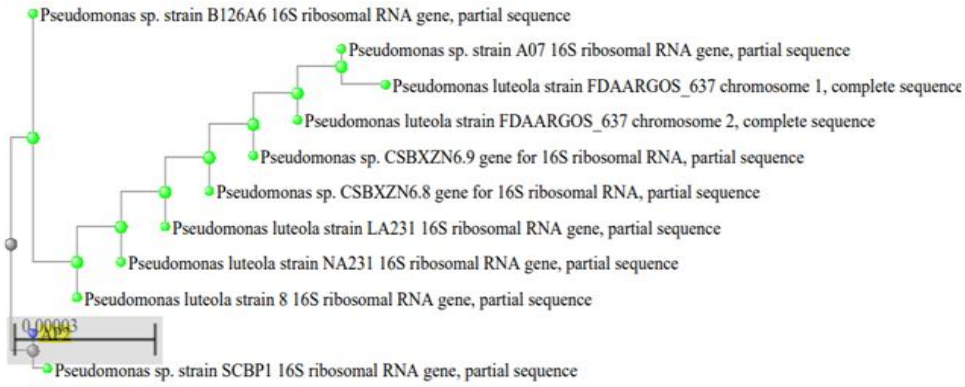

Fig-3: Phylogenetic tree of selected bacterial isolate

3.3 ICP-MS analysis of untreated and treated HDS

The screening of powdered catalyst sample and the characterization of its waste using inductively coupled plasma mass spectrometry (ICP-MS) at a high temperature of approximately 700° C. the catalyst waste sample contained magnesium (Mg^{+}) at a concentration of 1400 ppm initially, and after treatment with biosurfactant producing bacteria, the magnesium concentration was reduced to 750 ppm.

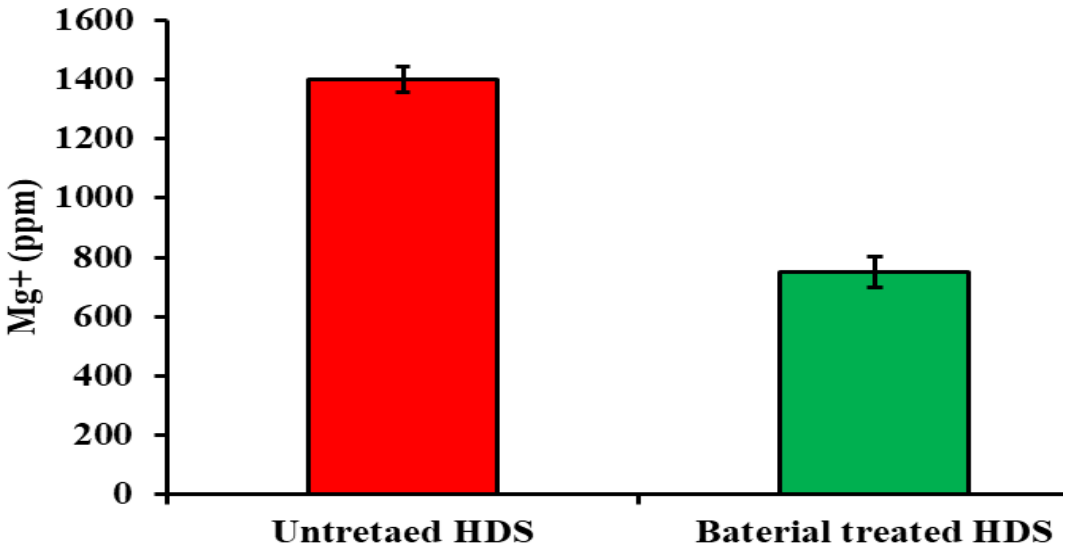

Fig-4: ICP-MS of untreated and bacterial treated HDS.

4. Conclusions

Magnesium is not only essential for humans but also for animals and plants. It is a naturally occurring element found abundantly in the Earth's crust and is essential for all cellular life. It is a vital component of various enzymes and plays a significant role in cellular energy production. So for environmental and economic benefits, the metal extraction technology proposed in the above study using biosurfactant-producing bacteria can offer an eco-friendly approach to recover and reutilize heavy metals from waste materials. This has potential environmental benefits by reducing the environmental impact of waste disposal and conserving natural resources. Additionally, the recovery of valuable metals can have economic advantages by providing a sustainable source of these metals.

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