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Bioaccumulation and Biosorption of Mercury using Indigenous Bacteria from Industrial Effluent

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A B S T R A C T

Heavy metal pollution in soils is a significant global environmental issue due to the toxic effects of metals and their accumulation in the food chain. Increased industrial activities have elevated heavy metal levels, necessitating effective remediation methods. Biosorption is an efficient, cost-effective, and eco-friendly technique for addressing heavy metal contamination. This study focused on the biosorption of Hg (Mercury) using bacterial isolates from polluted soils. Biosorption rates were determined using atomic absorption spectrophotometry. Isolated cultures were incubated Luria Bertani media with varying metal concentrations for 5 days. SG 2 (*BRucella anthropi*), SG 10 (*Bacillus pumilis*), and SG 14 (*Staphylococcus edaphicus*) showed the highest biosorption rates of 97.59%, 97.58% and 97.62% for Hg. These results indicate the potential of *BRucella anthropi*, *Bacillus pumilis*, and *Staphylococcus edaphicus* for biosorbing Hg, highlighting their usefulness in remediating contaminated soils.

Introduction

Hg is a toxic heavy metal and is considered one of the "ten leading chemicals of concern" by the World Health Organization (WHO). It cycles through the atmosphere, water, and soil in various forms, becoming a global environmental issue. Awareness programs have been organized under an international action plan to minimize Hg emissions and remediate pollution, which is introduced into the environment both naturally and through human activities. This creates a significant threat to both human health and wildlife, even in areas that may not appear overtly polluted (Rehman *et al.,* 2007). The risk posed by Hg depends on several factors, including exposure likelihood, the chemical form of Hg (as some forms are more toxic), and the geochemical influences that affect its behavior in the environment. Hg is known to be a persistent, bioaccumulative pollutant that accumulates in water sediments and transforms into methylmercury, a more toxic form that readily enters the food chain. Once released into the environment, Hg can cycle back and forth between air and soil, maintaining its presence in the ecosystem without being removed, merely relocated into other forms or locations (Aryal, 2021). Methylmercury can easily enter the bloodstream and affect the brain, leading to serious neurological

issues. In aquatic ecosystems, it accumulates in algae, which are consumed by smaller fish, and then those fish are eaten by larger ones, resulting in a magnification of methylmercury concentrations up the food chain—large predatory fish like shark and swordfish can contain 100,000 times more methylmercury than the surrounding water.

Although methylmercury is seldom found in drinking water, it poses significant risks through fish consumption, leading to various health disorders in humans (Aryal, 2021). It is advisable to eat fish with lower mercury levels, such as shrimp, light canned tuna, salmon, and catfish. Natural sources account for about 30% of atmospheric Hg emissions, such as volcanic eruptions and forest fires, while about 70% comes from anthropogenic activities, including the mining of mercury ores and the burning of fossil fuels.

Hg can also re-enter the environment via the evaporation of contaminated water (François *et al.,* 2012). When released, Hg may travel hundreds of miles with the wind before settling on land. Once deposited, it can enter waterways through rain and runoff, contaminating lakes and streams. Various industrial processes, agricultural activities, and even household products contribute to Hg emissions.

For instance, elemental mercury vapor can be harmful to the nervous system in high concentrations. Major contributors to these emissions include the chlor-alkali industry, coal-based thermal power plants, cement production, thermometer manufacturing, and waste from hospitals and municipalities. Mining is one significant source of mercury contamination. Large amounts of liquid Hg are used to concentrate precious metals like gold and silver, leading to Hg loss into the environment during the mining and recovery processes, and this practice continues in some countries to this day.

Additionally, inorganic Hg, found in batteries and produced during the manufacture of chlorine, can contaminate water sources. While it is more commonly found in drinking water, its harmful effects primarily manifest in the kidneys. Methylmercury, produced from elemental Hg by aquatic organisms, enters fish and shellfish, posing serious risks to the human food chain. Consequently, guidelines recommend that vulnerable populations, such as pregnant women and young children, avoid high-mercury fish types like shark, swordfish, and king mackerel (Akkoyun *et al.,* 2020).

Heavy metal pollution in soils is a serious global issue, driven by industrial activities, necessitating effective remediation methods. Biosorption is a cost-effective and eco-friendly approach to combat heavy metal contamination (De *et al.,* 2014). This study focused on biosorbing Hg using bacteria isolated from polluted soils. Biosorption rates were determined using atomic absorption spectrophotometry over 5 days.

Staphylococcus edaphicus achieved the highest rates of 97.62% for biosorption of Hg. These findings highlight the potential of the bacteria in remediating contaminated soils.

Materials and Methods

Sample collection

Soil samples contaminated with heavy metals were collected from various regions in Karnataka, India, as shown in Table I. The samples were placed in clean, sterile polythene bags and processed within 24 hours of collection (Das and Choudhury, 2016).

Isolation and Screening for biosorption of heavy metal resistant bacteria

To isolate Hg -resistant bacteria, LB (Luria-Bertani) agar media was prepared by incorporating Hg (using an analytical standard from the National Institute of Standards and Technology (NIST)) at concentrations of 5 mg/ml, 10 mg/ml, and 12.5 mg/ml.

A 1 ml aliquot of soil sample diluents from dilutions 10^{-5} and 10^{-6} was aseptically inoculated into the media using the pour plate method and then incubated at 37°C for 24 hours (Oyewole *et al.,* 2019). The resistant bacterial colonies that developed were subsequently picked and stored.

Biomass & Bioaccumulation

Bacteria were grown in LB broth containing 10 mg/L of the Hg and incubated at 37°C for 48 hours. The bacterial cells were harvested by centrifugation at 6000 rpm for 15 minutes and then suspended in 1 mL of distilled water. The suspension was oven-dried and weighed.

The dried biomass was then digested by adding 5 mL of concentrated nitric acid. The beaker containing the mixture was placed on a hot plate, stirred continuously,

and heated until nitrogen oxide fumes were released and a white residue remained. After cooling to room temperature, the solution was diluted to 50 mL with distilled water. The mercury concentrations in the supernatant were analyzed using an atomic absorption spectrophotometer (AAS) (Thermo Scientific ICE 3000 Series AA Spectrometer) at a wavelength of 600 nm to determine the heavy metal concentration (Rahman *et al.,* 2019). The percentage of biosorption was calculated using the following formula

$$(\%)\ \text{biosorption} = \frac{\text{initial metal concentration-final metal concentration X 100}}{\text{initial metal concentration}}$$

Optimization studies for Biosorption and Bioaccumulation

Biosorption capacity in bacteria is influenced by factors like initial pH, temperature, inoculum size, and metal concentration. To optimize these parameters for enhanced biosorption, isolates were tested at temperatures of 25, 30, 35, and 40°C. The pH varied from 6 to 10, and inoculum sizes ranged from 0.25 to 1.25 ml (Das and Kumari, 2016).

DNA extraction and PCR amplification of 16S rRNA genes

DNA was extracted from the isolates SG2, SG10, and SG14 using the method of Ferrari and Hollibaugh (Ferrari and Hollibaugh, 1999). The DNA was rehydrated in 50 ml of sterile deionized water and subjected to PCR amplification with universal 16S rRNA primers: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Swamy *et al.,* 2016). Each 50 ml PCR mixture contained 10 mM Tris–HCl (pH 8), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.5 pmol of each primer, 1.25 U of Taq polymerase (Sigma–Aldrich), and 10 ng of DNA template (Sambrook *et al.,* 1989).

The PCR was performed in an ABI thermal cycler with a denaturation step at 95 °C for 5 minutes, followed by 35 cycles of 1 minute at 94 °C, 1 minute at 55 °C, and 2 minutes at 72 °C, and a final extension at 72 °C for 10 minutes. Amplification was confirmed using gel electrophoresis. PCR products were purified with the Mo Bio 250 Prep Cleanup Kit and sequenced with an ABI 3130XL genetic analyzer. The 16S rRNA sequences were compared to those in GenBank NCBI using the BLAST server, and multiple sequence alignments and phylogenetic tree construction were done with MUSCLE in Mega 6.00 (Sambrook *et al.,* 1989). The nucleotide sequences of the three isolates were deposited in NCBI/GenBank.

Results and Discussion

Screening and selection of Hg resistant bacteria

Bacterial isolates that are resistant to mercury (Hg) were screened from various soils contaminated with heavy metals. The evaluation of these isolates for their biosorption potential revealed that isolates SG2, SG10, and SG14 exhibited the highest biosorption rates of 97.59%, 97.58%, and 97.62%, respectively, when grown on Luria-Bertani (LB) medium supplemented with Hg. The results are illustrated in Fig. I.

Optimization parameters for the biosorption and bioaccumulation of bacteria

Optimization of pH

The effect of pH on the biosorption of Hg was examined at various pH levels: 6, 7, 8, 9, and 10. After inoculation, the tube sets were incubated at 37°C, and the growth of the isolates was measured using a spectrophotometer at 660 nm. Notably, SG10 demonstrated the highest biosorption percentage for Hg, achieving 98.08% at pH 7 (Fig. II).

Optimization of temperature

The Hg -resistant isolates were inoculated into 100 ml of LB broth containing Hg and incubated at different temperatures of 25°C, 30°C, 35°C, and 40°C for 24 hours. SG10 exhibited the highest biosorption percentage of 98.14% at 35°C for Hg (Fig III). The optical density was measured to assess growth in terms of turbidity using a spectrophotometer at a wavelength of 660 nm.

Optimization of inoculum size

The effect of inoculum size on the biosorption of Hg was examined at various levels: 0.25, 0.50, 0.75, 1.0, and 1.25 ml. The highest percentage of biosorption (98.2%) for Hg was observed with the SG14 strain at an inoculum size of 1.0 ml, measured at 660 nm (Fig. IV).

Molecular characterization and phylogenetic tree analysis.

The genomic DNA of all three Hg -resistant strains was successfully extracted, allowing for the amplification of the 16S rRNA gene in each of them. Complete contig sequences of three bacterial isolates were then analyzed using BLAST (Basic Local Alignment Search Tool) against the NCBI database. The results showed that the Hg-resistant bacterial isolate SG2 shared 95% similarity with *BRucella anthropi*, SG10 had 99% similarity with *Bacillus pumilus*, and SG14 displayed 96% similarity with *Staphylococcus edaphicus* (Fig V). The 16S rRNA sequences of these three heavy metal-resistant bacterial isolates have been deposited in the NCBI/GenBank database under the accession numbers PQ056703, PP994963, and PP994964.

Environmental pollution, particularly involving heavy metals, is a significant concern due to its adverse effects on human health. Certain types of microbial biomass exhibit biosorption properties for metallic ions, which are often found in environments contaminated by metals. The presence of these contaminants exerts selective pressure, enabling microbes to survive and adapt through mechanisms that counteract the effects of heavy metals. This study demonstrates the potential of microorganisms to address metal contamination by utilizing their ability to remove metals from polluted soil through the biosorption process. Heavy metal-resistant bacteria were isolated from industrial effluents collected from various regions of Karnataka, India. Among the isolated organisms, only three exhibited a very high resistance to Hg, with minimum inhibitory concentrations (MIC) for heavy metals ranging from 5 mg/ml to 12.5 mg/ml (Nwagwu *et al.,* 2016).

The study also examined the influence of the initial concentration of heavy metals to establish the relationship between the starting concentration of metal ions and the biosorption capacity (Ma *et al.,* 2015). In the early stages of biosorption, an increase in heavy metal concentration correlates with an enhancement in biosorption. At lower concentrations, the bacterial surface has more available binding sites for metal ions. However, at higher concentrations, these sites tend to become saturated (Ren *et al.,* 2015). Furthermore, the ratio of metal ions to the available surface area is higher at low initial metal concentrations, while at high concentrations, fewer sites remain accessible for binding with metal ions. Earlier studies have shown that *Bacillus subtilis* exhibited the highest MIC at 0.05 mg/ml (Sing *et al.,* 2014), while *Serratia marcescens* had the highest MIC at 0.001 mg/ml (Francois *et al.,* 2012). Additionally, Lactobacillus casei displayed an MIC of 0.05 mg/ml (Rehman *et al.,* 2007), and *Pseudomonas aeruginosa* had an MIC of 0.04 mg/ml for mercury. Compared to these related studies, the organisms isolated in our study demonstrated significantly higher MIC values (up to 12.5 mg/ml) and therefore show greater potential for biosorption studies. Among these, *Staphylococcus edaphicus* exhibited the highest resistance against mercury.

The concentration of hydrogen ions is a crucial factor in the removal of heavy metals (Tasar *et al.,* 2014). pH plays a significant role in determining the solubility of metals and the functionalization of microbial sorbent surfaces (Morosanu *et al.,* 2017). A study by Colak *et al.,* (2011) and Rehman *et al.,* (2007) found that the biosorption capacity for mercury (Hg) was highest at pH 7.5, while the absorption capacity for *Rheinheimera aestuarii* was greatest at pH 6 (Baek and Jeon, 2015). In our current study, the maximum uptake of metal ions occurred at pH 7. As pH increased beyond this point, metal absorption declined gradually. A similar trend was observed in bacterial biomass, with reduced growth rates in both highly acidic and alkaline conditions. Therefore, maintaining an optimal pH is critical as it promotes active microbial metabolism, which is necessary for survival and effective sorption (Choi *et al.,* 2009). Temperature also significantly affects the biosorption of heavy metals. In the study by Yao *et al.,* (2023), Bacillus sp. exhibited the highest biosorption capacity for Hg at 30°C. In the present study found that the ideal temperature for bacterial growth was 35°C, making it easier to maintain the isolates for in situ bioremediation. The inoculum size of heavy metal-resistant bacteria is essential for determining their effectiveness in biosorption and bioaccumulation. A larger inoculum size enhances the bioavailability of heavy metals through mechanisms such as biosorption, bioaccumulation, and biotransformation. Conversely, a smaller inoculum size prolongs the time required for effective remediation (Bidlas *et al.,* 2008). Our study determined that the optimum inoculum size was 1 ml for all the isolates at a wavelength of 660 nm. The potential biosorbing bacteria identified were *Rucella anthropi* (SG2), *Bacillus pumilus* (SG10), and *Staphylococcus edaphicus* (SG14).

Figure.1 % of biosorption of bacterial isolates

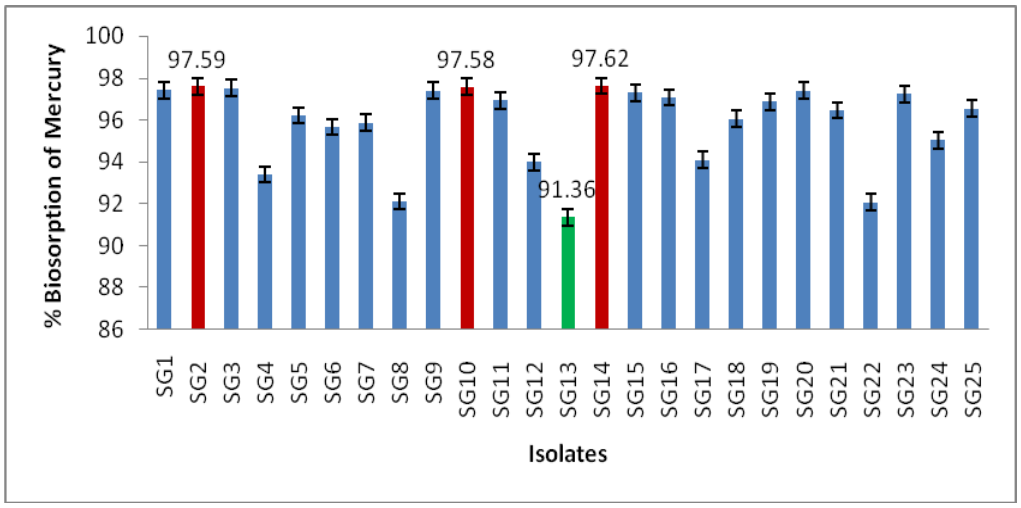


Figure.2 Optimization of pH for selected bacterial isolates

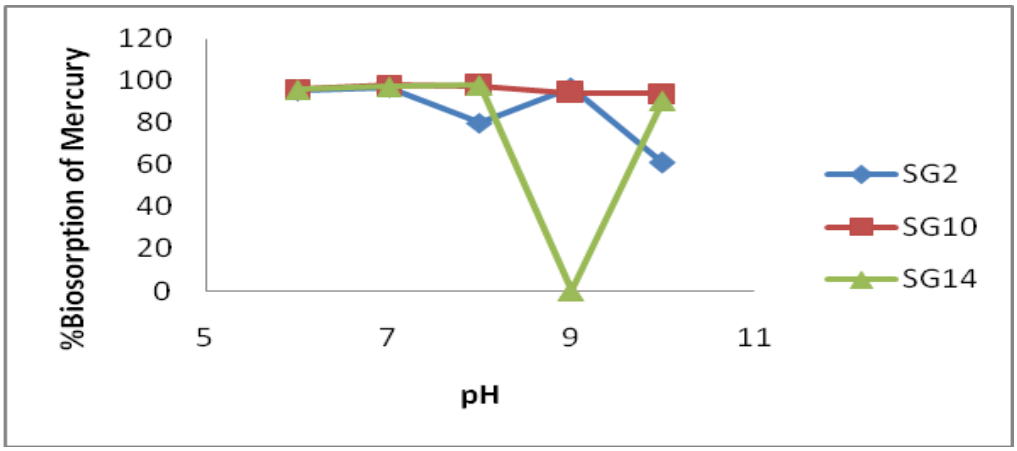


Figure.3 Optimization of Temperature for selected bacterial isolates

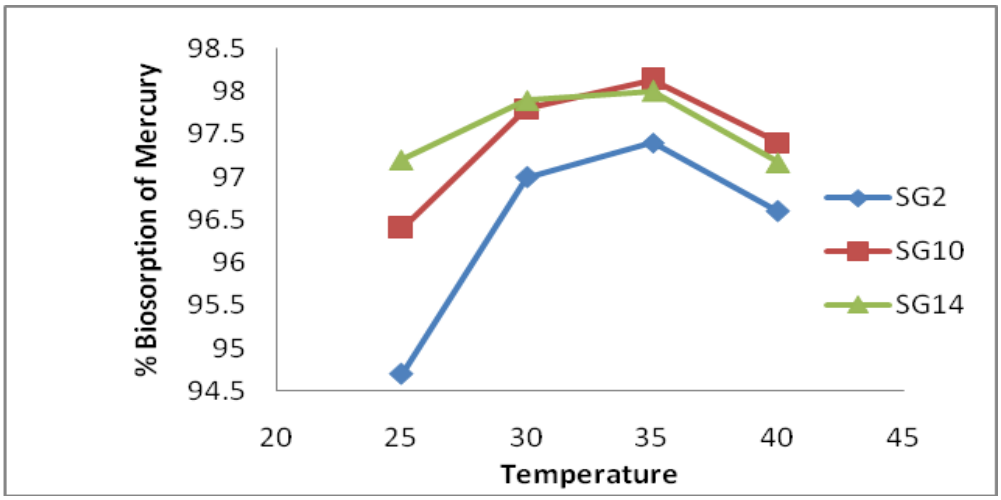


Figure.4 Optimization of inoculums size for selected bacterial isolates

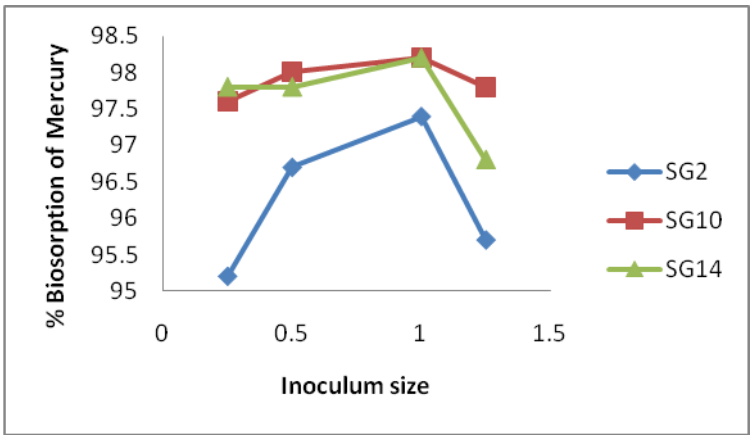
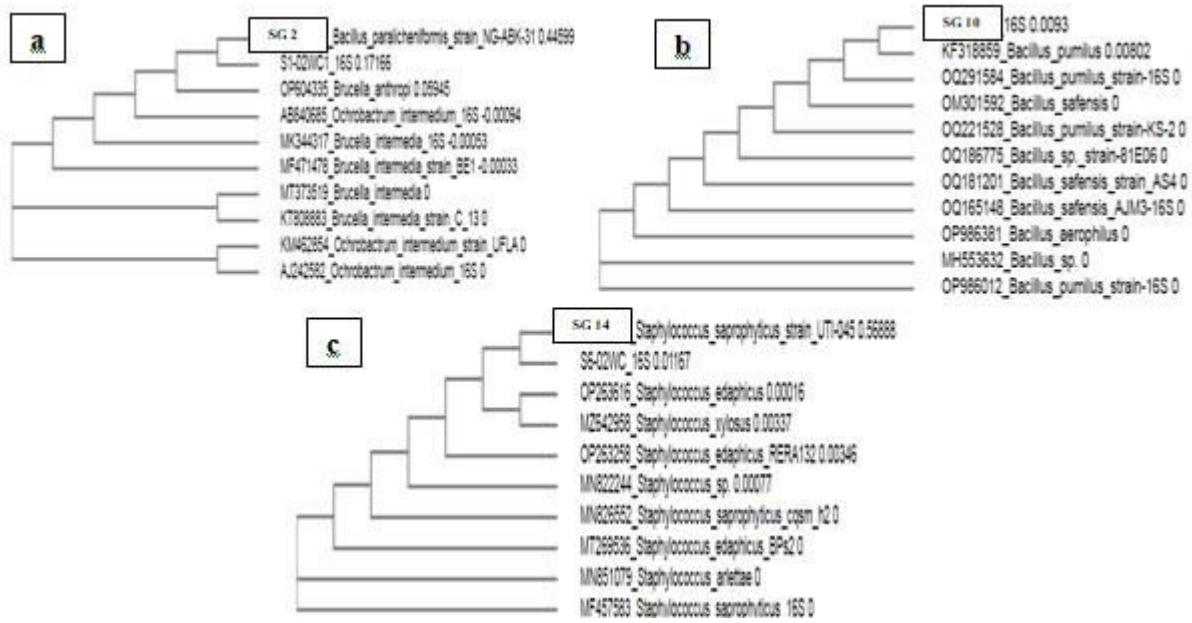


Figure.5 Phylogenetic tree of selected bacterial isolates a) *Brucella anthropi* b) *Bacillus pumilis* c) *Staphylococcus edaphicus*



Evidence of Hg biosorption by *Staphylococcus edaphicus* is scarce, making it a novel species identified for this purpose. Given these findings, we consider these three organisms to be promising for reducing the accumulation and biosorption of heavy metals in the soil.

The study demonstrated that bacterial strains sourced from contaminated soils exhibited varying levels of metal tolerance. The biosorption of Hg heavy metals by three bacterial biosorbents SG2, SG10, and SG14 was highest at the initial metal concentration, followed by a gradual decline. The optimum temperature for metal biosorption was found to be 35°C, with the most effective metal removal occurring at a pH of 7. Additionally, the ideal inoculum size for biosorption was determined to be 1 ml. Moreover, these strains were able to significantly reduce metal toxicity through biosorption, though the effectiveness varied based on factors such as metal concentration, pH, temperature, and inoculum size. Specifically, the bacterial strains *BRucella anthropi* (SG2), *Bacillus pumilus* (SG10), and *Staphylococcus edaphicus* (SG14) demonstrated the ability to biosorb Hg when cultured under metal-stressed conditions. These findings suggest that these metal-tolerant bacterial strains

could serve as a cost-effective and eco-friendly solution for remediating heavy metal-polluted soils, thereby making them suitable for crop cultivation.

Author Contributions

Shilpa Hosmani: Investigation, formal analysis, writing—original draft. Gayathri Devaraja: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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