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Bioremediation of Toxic Heavy Metals Cr (VI) from Tannery Effluent using Micro-Organisms: Biotechnological Potential

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Abstract

Increased industrialization and human activities have impacted on the environment through disposal waste containing heavy metals. Heavy metals pollution of agricultural soil has been mainly due to the disposal of industrial wastewater, sewage and sewage sludge to agricultural land. Conventional methods for the removal of heavy metals from aqueous solutions are not economically and environmental friendly because it has produced massive quantity of toxic chemical compounds. Naturally fungi have a large variety of extracellular proteins, organic acids and other metabolites. Fungi can adapt in any ecosystems and any environmental conditions Interest in processes involving heavy metal uptake by microorganisms has increased considerably in recent years due to the biotechnological potential of micro-organisms in removing and/or recovery of metals.

1. Introduction

Global pollution is increasing due to the variations in the natural and anthropogenic activities leading to contamination of various terrestrial and aquatic ecosystems with heavy metals, inorganic and organic compounds and radionuclides. Controlled and uncontrolled discharge of solid and liquid wastes, accidental spillages, and use of agricultural fertilizers, herbicides, insecticides, weedicides, sewage disposal, explosives and tar are some of the main culprits of exponentially increased content of various contaminants in the biosphere.

Basically, our environment is composed of atmosphere, earth, water and space. The interaction of the atmosphere, lithosphere, hydrosphere and biosphere is continuing for years together. On account of the various activities of man, the composition and complex nature of environment gets changed. These activities include industrialization, construction, and transportation. Such activities, although desirable for human development and welfare, lead to generation and release of objectionable materials into the environment thus turning it foul and rendering our life miserable. The natural environment is clean, but due to multifarious activities of man it gets polluted resulting in what is called as environmental pollution. In absence of pollution, it remains clean and enjoyable.

Our main aim should be to keep our environment clean, by curbing industrial activities. However, in order to keep pace with the rapid industrialization world over, a developing country like India cannot afford to arrest its industrial growth. We can select such industrial processes, which would cause minimum or zero pollution. Further, it is necessary to undertake pollution control measures, so as to enable us to keep our environment as clean as possible.

Amongst the developing countries, India is the foremost nation to take note of degradation of environment. In 1972, in the world environmental conference held at Stockholm, our government declared that it was its responsibility to control pollution in India. Even during the global conference held at Kyoto in Japan, India accepted the responsibility of reducing global warming and depletion of ozone layer. Perhaps India is the foremost country in third worlds to implement rigorously pollution legislation both for air and water. India has accepted the stipulated levels of the pollutants in act designated as 'maximum permissible level'.

2. Tannery Industry and its Effluents

Tanning is the process by which raw animal hides are converted into leather. During this process, the leather is made resistant to biological decay by stabilizing the collagen structure of the hide, using natural or synthetic chemicals (UNEP, 1991).

The tannery industry represents an important sector in the economy of many countries. Disagreeable odor emanating from the decomposition of protein solid waste, presence of hydrogen sulphide, ammonia etc. are normally associated with tanning activities. Depending on the leather process, it generates large quantities of wastewater with ammonium, sulfates, surfactants, acids, dyes, sulfonated oils, chlorinated phenols, chromium (Mwinyihija, 2007) and organic substances, including natural or synthetic tannins. These chemical substances are applied to transform the animal skin into products with great capacities for dyeing, as well as to increase the mechanical and hydrothermal resistance. Considering that the greater part of these organic compounds are resistant to conventional chemical and biological treatments, the wastes discharged into natural waters increase environmental pollution and the health risks (Song *et al*., 2000). The treatment of this type of wastewater is very complex mainly because of the variety of chemical products added in different concentrations (Preethi *et al*., 2009).

Other pollutants from tanneries to be considered are Azodyes, Cadmium compounds, Cobalt, Copper, Antimony, Barium, Lead, Selenium, Mercury, Zinc, Arsenic, Nickel and Polychlorinated Biphenyls (PCB). Liquid effluent from light leather processing contains organic matter, chromium, sulphide, while solid waste includes fleshing, wet blue splits, trimmings and shavings and buffing dust.

The leather industry in India is geographically well diversified, though Tamil Nadu, Uttar Pradesh and West Bengal account for bulk of the output. The major production centers for leather and leather products are located at Chennai, Ambur, Ranipet, Vaniyambadi, Trichi, Dindigul in Tamil Nadu, Kolkata in West Bengal, Kanpur, Agra and Noida in Uttar Pradesh, Jalandhar in Punjab, Bahadurgarh and Manesar in Haryana, Bangaluru in Karnataka, Telangana and Delhi. The sector is dominated by micro and small units with bigger units accounting for just around 5 per cent of the total manufacturing units.

The tannery industry mushrooming in North India in cities like Kanpur, Unnao, Agra and Noida, has converted the Ganga River into a dumping ground. The tanning industry discharges different types of wastes into the environment, primarily in the form of liquid effluents containing organic matters, chromium, sulphide, ammonium and other salts. As per an estimate, about 80-90% of the tanneries use chromium as a tanning agent. Of this, the hides take up only 50-70%, while the rest is discharged as effluent. Pollution becomes acute when tanneries are concentrated in clusters in small area like Kanpur. Consequently, the leather-tanning sector is included in the Red category of industries due to the potential adverse environmental impact caused by tannery wastes.

3. Discharge of Industrial Effluents into the Ganga

Study on the distribution of heavy metals in sediments of the river Ganga and its tributaries have been carried out by several workers. Monitoring of Ganga River from Rishikesh to Varanasi indicated that Kannauj to Kanpur and Varanasi are the most polluted stretches of the river Ganga. Analysis of upstream and downstream water and sediment revealed a 10-fold increase in chromium level in the sediment at downstream Jajmau area of Kanpur showing unchecked release of untreated tannery effluent.

3.1. Volume of industrial effluents discharged into the Ganga

The Comptroller and Auditor General (CAG) report on Ganga action plan dated December 11, 2000 had estimated that about 2,667 mld industrial effluents were discharged into the river. Public accounts committee's 62nd report gives some estimates of industrial pollution as on 2003. According to this report the industrial wastewater generation amounted to 2789 mld. This is despite closing down a number of polluting industries. This does not include the industrial effluents from Uttar Pradesh.

3.2. State of common effluent treatment plants (CETPs) in the Ganga basin

Total CETP Capacity: 191 mld [Delhi (133 mld) + Kanpur (36 mld) + Unnao (2 mld) + Mathura (6.25 mld) +Kolkata (20 mld installed)].

4. Heavy Metals

Heavy metals, broadly defined as a group of toxic metals and metalloids associated with pollution and toxicity. They are elements with a density of more than 60 mg m^{-3} and atomic weights that exceeded that of iron (Fe) (Fenglian and Wang, 2011). Although their natural presence is limited to a few soil habitats, they are locally present as a consequence of human activities including mining, processing or the extensive use of the metal. Unlike organic pollutants, heavy metals are not biodegradable and thus pose a serious problem requiring remediation (Guria *et al*., 2014). Since the presence of heavy metals interferes with many physiological processes, they affect the whole ecosystem (Lokhande *et al*., 2011; Thirumurugan *et al.,* 2017) and can have effects that range in scale from a single cell to the whole community.

Today heavy metals are abundant in our drinking water, air and soil due to our increased use of these compounds (Chaitali and Dhote, 2013). They are present in virtually every area of modern consumerism from construction materials to cosmetics, medicines to processed foods; fuel sources to agents of destruction; appliances to personal care products. It is very difficult for anyone to avoid exposure to any of the many harmful heavy metals that are so prevalent in our environment (Raman *et al*., 2018).

5. Toxicological Aspects of Heavy Metals

Due to their mobility in aquatic ecosystems and their toxicity to higher life forms, heavy metals in surface and groundwater supplies have been prioritized as major inorganic contaminants in the environment. Even if they are present in dilute, undetectable quantities, their recalcitrance and consequent persistence in water bodies imply that through natural processes such as bio magnification, concentrations may become elevated to such an extent that they begin exhibiting toxic characteristics.

These metals can either be detected in their elemental state, which implies that they are not subject to further biodegradative processes or bound in various salt complexes. In either instance, metal ions cannot be mineralized. Organic and exchangeable forms of heavy metals are the major forms taken up by plant species (Alva *et al*., 2000). In contaminated environments, certain plant species can accumulate considerable amounts of heavy metals and protect the soil from erosion and the leaching of heavy metals into the groundwater (Vangronsveld *et al*., 1996; Raman *et al*., 2018). The toxic effects of heavy metals are manifested in a wide range of plant cellular activities including photosynthesis, respiration, mineral nutrition and membrane structure in all the plant groups (Garty, 2001). Toxic concentrations have been found to cause membrane damage, ion leakage and decreased chlorophyll concentrations in vascular plants (Patsikka *et al*., 2001), as well as in bryophytes (Guschina and Harwood, 2002) and lichens (Hyvarinen *et al*., 2000). Apart from environmental issues, technological aspects of metal recovery from industrial waters must also be considered.

6. Sources of Heavy Metals and Their Effects on Human Health

Natural weathering of metalliferous rocks and anthropogenic sources provide the two major inputs of metals into ecosystems (Ross, 1994). Natural sources of these elements in the atmosphere are volcanic activity, terrestrial dust, vegetation fires, salt spray from the oceans and biogenic sources (Nriagu, 1989). Anthropogenic sources in the soil-plant systems include atmospheric deposition originating from a range of industrial activities (metalliferous mining and smelting, alloying plants, petrochemical industry, fertilizer plants, coal power plants, industrial and home furnaces), agricultural amendments and motor traffic (Alloway, 1990). The amount of heavy metals emitted by natural processes into the atmosphere is small compared with the anthropogenic input of these elements (Ross, 1994). Heavy metal deposition shows a high affinity for adsorption to organic surfaces. Heavy metals in soils can exist in water soluble and exchangeable form, associated with insoluble organic matter, and as carbonates, oxides of Fe, Al and Mn, and layer silicates (Alloway, 1990). Generally, increasing acidity tends to increase the mobility and toxicity of heavy metals (Raman *et al.*, 2018).

The heavy metals hazardous to humans include lead, mercury, cadmium, arsenic, copper, zinc, and chromium. Such metals are found naturally in the soil in trace amounts, which pose few problems. When concentrated in particular areas, however, they present a serious danger (He *et al*., 1992).

7. Need for the Removal of Heavy Metals

Continuous discharge of industrial, domestic and agricultural wastes in rivers and lakes causes deposit of pollutants in sediments. Such pollutants include heavy metals, which endanger public health after being incorporated in food chain. Heavy metals cannot be destroyed through biological degradation, as is the case with most organic pollutants. Incidence of heavy metal accumulation in fish, oysters, sediments and other components of aquatic ecosystems have been reported from all over the world (Ahalya *et al*., 2005; Raman *et al.*, 2018).

8. Conventional Methods for the Treatment of Metals

Over the last few decades, several methods have been devised for the treatment and removal of heavy metals. Numerous industries discharge a variety of toxic metals into the environment. For several years now, it is mandatory that industry is required to remove metal pollutants from liquid discharges. Several physico-chemical methods like chemical precipitation, electrodialysis, ion-exchange, ultra-filtration, reverse osmosis etc. are commonly employed for stripping toxic heavy metals from waste waters (Mehta and Gaur, 2001).

8.1. Bioremediation

The heavy metals can be removed by bioremediation. The process can be divided into two types:

8.1.1. Phytoremediation - i.e. removal by higher plants

The term 'phytoremediation' is a combination of two words: Greek *phyto* (meaning plant) and Latin *remedium* (meaning to correct or remove an evil). Green plants have an enormous ability to uptake pollutants from the environment and accomplish their detoxification by various mechanisms. Phytoremediation technology is a relatively recent technology with research studies conducted mostly during the last two decades (1990 onwards). The concept of phytoremediation (as phytoextraction) was suggested by Chaney (1983). Phytoremediation has low installation and maintenance costs compared to other remediation options (Van der Ent *et al.,* 2103). Regarding cost, phytoremediation can cost as less as 5% of alternative clean-up methods (Prasad, 2003). The method of phytoremediation exploits the use of either naturally occurring metal hyper accumulator plants or genetically engineered plants (Setia *et al*., 2008). Olguin and Galvan (2010) stated that a variety of polluted waters can be phytoremediated. A rising method for polluted area remediation is phytoextraction (Ok and Kim, 2007), the uptake of pollutants by plant roots and translocation within the plants state that pollutants can be generally removed by harvesting the plants (Singh *et al*., 2011). Plants may play a vital role in metal removal through absorption, cation exchange, filtration, and chemical changes through the root.

8.1.2. Microbial remediation

The ability of microbial biomass (bacteria, algae and fungi) to remove heavy metal ions from polluted aquatic systems has been reported and has also attracted much interest in recent years. The cell surface of algae used in biosorption is mainly covered by alginate and sulfated polysaccharides (Davis *et al*., 2003). The outer cell wall of fungi is mainly comprised of beta-(1_3) - glucan and glycoproteins whereas lipopolysaccharides and peptidoglycan with (lipo) teichoic acids are characteristic for gram-negative and positive bacteria, respectively. Concentrations of functional groups responsible for metal binding varies between biomasses (Chojnacka *et al*., 2005). In addition, different functional groups have different affinities for a given metal, leading to the varying removal capacities of different biomasses (Ngwenya *et al*., 2003). Different physical and chemical treatments have been tested to increase the metal removal capacity of biomasses, and for desorption of bound metals. The results have been variable and have depended on the treatment, biomass, and metal studied (Akar and Tunali, 2006). Desorption experiments using dilute mineral acids like (HCl, HNO_3 and H_2SO_4), have established the reversibility of heavy metal removal by algal, fungal, and bacterial biomass. Loofa sponge immobilized biomass of *Chlorella sorokiniana* (LSIBCS) was shown to be highly effective in removing Cr (III) from aqueous solution (Akhtar *et al.*, 2008).

9. Immobilized Biomass

Microbial biomass is often immobilized onto a suitable carrier material. Immobilization of the biomass is important, since it improves the mechanical strength of the biomass, and especially in the case of small biomass particles, reduces resistance to fluid flow. The most frequently used technique for biomass immobilization is entrapment within a polymer matrix such as sodium alginate, agar, silica gel, polyacrylamide, and polysulfone (Mehta and Gaur, 2005) but other methods such as adsorption on inert supports, covalent bonding to vector compounds and cross-linking of cells have been used (Vegliò and Beolchini, 1997). Metal removal by immobilized biomass is often lower than by free biomass. For example, nickel removal by *Pseudomonas fluorescens* decreased from 145 to 37 mg g^{-1} after immobilization on agar beads (Lopez *et al.,* 2002). The reduction in removal after immobilization depends on the matrix used, and the removal ratio of immobilized biomass/free biomass has been reported to vary from 0.92 to 0.42 for different matrices when applied to chromium binding (Bai and Abraham, 2003). This has been attributed to a reduction in the available surface area of the biomass as a result of immobilization. Chemical modifications and potentiometric titrations of biomasses have revealed that carboxyl, phosphoryl, sulfonate, and amino groups are the main functional groups responsible for heavy metal binding in bacteria, algae and fungi (Chojnacka *et al*., 2005).

10. Chromium

Chromium is a transition metal located in group VI-B of the periodic table. Cr is the seventh most abundant element on earth and 21st in the crystal rocks. Cr abundance in Earth's crust ranges from 100 to 300 μg g^{-1}. Soils may contain between 5 and 3000 μg of chromium per gram. The world production of Cr is in the order of 10^7 tons per year; 60–70% is used in alloys, including stainless steel, and 15% is used in chemical industrial processes, mainly leather tanning, pigments and electroplating. Its widespread use has converted Cr in a serious pollutant of air, soil and water. Cr concentrations in non-polluted waters vary from 0.1 to 0.5 ppm in fresh waters and from 0.0016 to 0.05 ppm in oceanic waters, but levels as high as 80 ppm have been observed in paper mill effluents. Although it is able to exist in several oxidation states, the most stable and common forms are the trivalent Cr (III) and the hexavalent Cr (VI) species, which display quite different chemical properties. Cr (VI), considered the most toxic form of Cr, is usually associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ions. In contrast, Cr (III) in the form of oxides, hydroxides or sulfates, is much less mobile and exists mostly bound to organic matter in soil and aquatic environments. Cr (VI) is a strong oxidizing agent and in the presence of organic matter is reduced to Cr (III); this transformation is faster in acid environments such as acidic soils. However, high levels of Cr (VI) may overcome the reducing capacity of the environment and thus persist as a pollutant. In addition, Cr (III) may be also oxidized to Cr (VI) in the presence of an excess of oxygen, being transformed again to the more toxic form.

10.1. Natural sources of chromium

Chromium is one of the earliest elements to be immobilized in solids by crystallization during differentiation of magma. Therefore it is normally concentrated in the ultramafic and ultrabasic rocks. Dunite, peridotite, pyroxenite, and serpentine contain Cr at levels of 2400 ppm (Matzat and Shiraki, 1974). The distribution of total Cr in natural ground waters was investigated by (Barnes and Langmuir, 1978). Chromium metal is an example of a heavy metal found in the effluents of industries, such as in metal finishing, petroleum refining, iron and steel industries, textile manufacturing, electroplating, leather tanning metal plating etc (Poornima *et al*., 2010). Effluents of these industries are chromium-laden. Hexavalent chromium Cr (VI) compounds are considered to be highly toxic, carcinogenic, and mutagenic to living organisms (Ramakrishna and Philip, 2005; Poopal and Laxman, 2009). But trivalent chromium Cr (III) is generally toxic only to plants at very high concentrations and is less toxic or non-toxic to human and animals (Megharaj *et al*., 2003).

10.2. Chromium transport and accumulation

Chromate is actively transported across biological membranes in both prokaryotes and eukaryotes. Once inside the cells, Cr (VI) is reduced to Cr (III) probably via the unstable Cr (V) and Cr (IV) states. In contrast, most cells are impermeable to Cr (III) probably because it forms water insoluble compounds in non-acidic aqueous solutions.

10.3. Chromium carcinogenicity

Chromium and most trivalent chromium compounds have been listed by the National Toxicology Program (NTP) as having inadequate evidence for carcinogenicity in experimental animals. According to NTP, there is sufficient evidence for carcinogenicity in experimental animals for the hexavalent chromium compounds; calcium chromate, chromium trioxide, lead chromate, strontium chromate, and zinc chromate. Because of its hazardous nature, toxicity and exposure, Cr (VI) has been designated as a priority pollutant in many countries (Megharaj *et al*., 2003). Due to the high toxicity of chromium the Central Pollution Control Board has fixed the maximum permissible limits of Cr (VI) and total chromium at 0.1 and 2 mg l^{-1}, respectively.

10.4. Chromium resistance in fungi

Chromium resistance has been described in filamentous fungi and yeasts. In the filamentous fungus *N. crassa*, chromate-resistant mutants induced by UV treatment were defective in sulfate transport. Previous observations linked a defect in sulfate uptake of *S. cerevisiae* to an increased resistance to chromate and selenate, ions that share the same permeases. It was proposed that this defect caused *S. cerevisiae* to down-regulate its sulfate uptake system; this lowered available sulfur led to the replacement of non-essential sulfur amino acids in proteins, resulting in significantly lower cysteine content compared to other eukaryotes. Yeast strains isolated from Cr-contaminated environments include the genus *Candida* (Ramirez *et al*., 2004), and to a minor extent *Rhodosporidium*. These yeasts are resistant to high concentrations of chromate as compared to the most common *S. cerevisiae*, which is more sensitive. In some species of *Candida* and *Rhodosporidium*, the general mechanism of chromate resistance is related to reduced ion uptake, rather than to biological reduction of Cr (VI) to Cr (III).

11. Bioremediation with Fungi

Yeasts and filamentous fungi offer a viable alternative for the bioremediation of waters and soils polluted by Chromium. One of the earliest references that fungi have good metal uptake system was by (Gadd, 1986). Fungi have an efficient metabolism independent biosorption. Fungal biomass have been found to be excellent biosorbents for sequestering heavy metals (Lewis and Kiff, 1988). The specific mechanisms of uptake differ quantitatively and qualitatively according to the species, the origin of the biomass and processing

(Tobin *et al*., 1984). The hyphal wall was found to be a primary site of metal ion accumulation (Tobin *et al*., 1984). This is attributed to several chemical groups (the acetamido group of chitin,amino, amido, sulphydryl and carboxyl groups in proteins and hydroxyls in polysaccharides)that might attract and sequester metal ions (Holan and Volesky, 1995). Biomass of fungi, such as *Absidia, Mucor* and *Rhizopus*, exhibit excellent metal-ion uptake (Venkateswerlu and Stotzky, 1989). Aquatic pycnidial fungi and aquatic Hyphomycetes like *Cylendrocarpon heteronemum* and *Penicillium viridicutum* are good biosorbents of trace metals and lead respectively (Khattab and Khatoub, 2007). *Trichoderma atroviride* is a potential candidate as a heavy metal biosorbent of zinc (Lopez and Vazquez, 2003). The yeast biomass has also been successfully used as a biosorbent for the removal of heavy metals such as Ag, Au, Co, Cu, Cd, Ni, Pb, U, Zn, and Th from aqueous solutions. Yeasts of the genera *Saccharomyces, Candida, Pichia* have proved to be efficient biosorbents for heavy metal ions (Gadd *et al*., 1988). In a study Mishra and Malik (2012) the effectiveness of a fungal isolate, *Aspergillus lentulus* FJ172995 for concurrent removal of heavy metals like chromium, copper and lead from industrial effluent was examined. Many fungal species such as *Rhizopus arrhizus, Penicillium spinulum* and *Aspergillus niger* have been extensively studied for heavy metal biosorption and the mechanism seems to be species dependent. Both alive and heat killed dead biomass of several filamentous fungi (*Mucor* spp., *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp.) have been employed (Ahmed *et al*., 2006, Thirumurugan *et al.*, 2017).

11.1. Chromium bioremediation by fungi

Many fungi contain chitin, the homopolymer of *N*-acetylglucosamine, and chitosan, a heteropolymer of *N*-acetylglucosamine and glucosamine, as an integral part of their cell wall structure. The deacetylated amino groups of glucosamine may act as binding sites for metals. In addition, a new siderophore type, named rhizoferrin, which shows increased Cr (III) biosorption, has been identified in Mucorales.Rhizoferrin is a polycarboxylate siderophore that is able to bind Fe (III), Cr (III), and Al (III).

Trivalent Cr and Al should not be reduced under physiological conditions. These metals remain bound to the siderophores and may be accumulated by the cells in a further stage; for some microorganisms this can be a mechanism of resistance to toxic metals.

Chemically treated mycelia from *Mucor mucedo* and *Rhizomucor miehei* efficiently bind Chromium (VI). The biomasses obtained from *Rhizomucor arrhizus*, *Candida tropicalis* and *Penicillium chrysogenum* are excellent biosorbents of Chromium. *Aspergillus carbonarius* NRC401121 adsorbs Chromium from solution. *Mucor hiemalis* MP/92/3/4 is also able to accumulate substantial amounts of Chromium Binding of Cr (III) by *M. hiemalis* is mainly a passive biosorption to the cell wall, whereas for the uptake of Cr (VI) intracellular accumulations as well as biosorption are possible. *S. cerevisiae* and *Candida utilis* have the ability to sorb Cr (VI) and the sorption capacity of dehydrated cells is considerably higher than that of intact cells. Chromate-resistant strains of *Aspergillus* spp. (Acevedo *et al*., 2008) and *Candida* spp., isolated from Cr-polluted environments, have shown Cr (VI)-reducing activity. *Bacteria as well as fungi* can be used as a potent bioremediation agent for treating tannery and industrial effluent in an eco-friendly process (Thirumurugan *et al.*, 2017).

12. Conclusion

The application of chromium in various manufacturing processes such as wood preservation leather tanning, electroplating, metal cleaning and processing, textile, ceramics and photographic sensitizer manufacturing has led to release of this metal into the environment. About 80% of the tanneries are engaged in chrome tanning processes and they use nearly 40,000 tons of basic chromium sulphate every year. At high concentrations Cr (III) salts used in tanning process exhibits toxic and carcinogenic behavior to the flora and fauna of the aquatic system. Bioremediation is measured to be very safe and obliging technology as it depend on microbes that occur naturally in the soil and pose no hazard to environment and the people living in that area. The fungal population in the system could also have developed resistance to several undesirable conditions and maintains physiological traits that could benefit microbial survival and maintenance in contaminated environments.

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