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BIOSURFACTANTS: APPLICATIONS AND ORGANISMS THAT PRODUCE THEM

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ABSTRACT

Biosurfactants which are amphiphilic compounds synthesized from microorganisms and plants have properties such as low toxicity and high biodegradability and are great alternatives in domestic and industrial uses. A lot of research has gone into production, characterization and uses of biosurfactants because of its eco-friendly properties in remediation of the environment. Crude oil and its products causes deleterious harm to the environment which needs to be cleaned up with environmentally friendly substances such as biosurfactants, so as not to cause more harm to the environment in the bid to get it cleaned up. Biosurfactants are cheaply available because the substrates used in their production are low cost and readily available. Biosurfactants display an accomplished and well-ordered application in various ways such as food, cosmetic, pharmaceutical, petroleum and agricultural industries. This review describes synthetic and biosurfactants classification, mechanism of action and applications. It also focuses on organisms that produce biosurfactants and why they do.

Keywords: Surfactants, Biosurfactants, Amphiphilic, Ecofriendly, Environment, Heavy metals

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INTRODUCTION

Surfactants have a remarkable ability to influence the properties of surfaces and interfaces; this has contributed to its large number of applications and wide use. Surfactants are amphiphilic substances containing a polar group (the hydrophilic part) which consists of O, S, P or N contained as part of functional groups such as alcohol, thiol, ether etc and an apolar group (the hydrophobic part) which is generally an hydrocarbon chain belonging to the alkyl or alkylbenzene kind. It sometimes contains the halogen atoms and some nonionized oxygen atoms. The word surfactant is short for surface- active-agent, substances that exhibit superficial or interfacial activities. The term surfactant was first used by Antara Products in 1950. Surfactants rest at water-oil interface when the three are mixed, and is called an emulsion or microemulsion [1].

It is worth noting that all amphiphilic molecules do not behave in such manner, only those with a hydrophilic-lipophilic balance migrate to the surface or interface. This does not take place if the amphiphilic molecule is either too hydrophilic or too lipophilic whereby it stays in either of the phase. There are two kinds of surfactants, natural and synthetic. Natural surfactant as the name depicts are strictly taken from a natural source [2] while the synthetic undergo an organic synthesis.

A natural surfactant is defined as surfactant taken directly from a natural origin [2] since there is a general notion that natural products are safe [3]. Due to concerns about environmental conservation, natural surfactants are of unique interest and the use of surfactants that are biodegradable and non-toxic would be in accordance with the current trend that natural cosmetics are in high demand because they are biodegradable and non-toxic [4]. Surfactants which originated from natural products will therefore present reduced environmental problems when selected as versatile cosmetic ingredients because of their safety, colour, odour and purity [5]. Natural surfactants are therefore surface active compounds (SACs) produced from vegetables and animals, by extraction, precipitation or distillation. SACs of microbial descent (biosurfactants), gotten in fermentation processes [6].

Many of the commercial surfactants are synthesized chemically from petroleum products [7] also mostly used in washing operations yet can result to significant environmental problems with continued use [8]. The manufacturing of a wide range of synthetic surfactants from petroleum products has increased tremendously since the early years of the twentieth century. Previously, natural surfactants like saponins (glycolipids), phospholipids like lecithin and fatty acid salt (soap) were commonly used domestically and industrially [9]. Recently, by-products of different technologies are used as carbon source for microorganisms in producing biosurfactants. Some of which are; molasses, frying oil soap stock and animal fat [10].

Numerous microorganisms produce biosurfactants (especially during their growth on substrates which are immiscible with water). Biosurfactants are a class of surface active metabolites which are able to reduce surface/interfacial tensions due to their hydrophilic and hydrophobic structural components by gathering spontaneously at the air–water or water–oil interface. Biosurfactants are divided into five major classes of lipopeptides, glycolipids, phospholipids, neutral lipids, and polymeric compounds based on their chemical structures [11]. Many biosurfactant-producing microorganisms have been recently isolated and identified. They belong to: *Bacillus*, *Pseudomonas*, *Agrobacterium*, *Streptomyces*, and *Thiobacillus* that produces amino acids-containing biosurfactants; *Pseudomonas*, *Torulopsis*, *Candida*, *Mycobacterium*, *Micromonospora*, *Rhodococcus*, *Arthrobacter*, *Mycobacterium*, *Corynebacterium*, *Mycobacterium*, and *Arthrobacter* which produces glycolipids; producers of phospholipids and fatty acids are *Thiobacillus*, *Aspergillus*, *Candida*, *Corynebacterium*, *Micrococcus*, and *Acinetobacter* [12].

Biosurfactants are surfactants produced by microorganisms which have received general attention with regards to their biodegradability, low toxicity, ecological acceptability and availability from renewable sources [13]. Mutagenicity and toxicity profile of biosurfactant from *Pseudomonas aeruginosa* and chemical surfactants was compared by [14] he pointed out biosurfactant as non-toxic and non-mutagenic. Biosurfactants have various structures which is determined by the microorganism from which they were obtained, the substrates utilized in the bioprocess and the

fermentation specifications [15]. The physicochemical characteristics of biosurfactants can be affected by a wide variety of variables especially surface properties like emulsification, foaming properties and biological properties [16]. These variations in functions, therefore, allows for a variety of applications depending on the surface characteristics of the biosurfactants that are stable at extremes of pH, salinity and temperature [17].

CLASSIFICATION OF SURFACTANTS

Classification of surfactants is based on charged groups present in their head. If it does not have any charged groups over its head, it is termed a nonionic surfactant. An ionic surfactant carries a net charge on its head, more specifically called anionic when the charge is negative, cationic when positive. If a surfactant contains a head with two oppositely charged groups, it is termed amphoteric or zwitterion. Biosurfactants are assigned to a particular group based mainly on their chemical structure and source [18]. The hydrophilic head is usually amino acid, peptide, mono-, di- or polysaccharide while the hydrophobic tail is commonly saturated, unsaturated, linear, branched or hydroxylated fatty acid.

CLASSIFICATION BASED ON CHEMICAL COMPOSITION

Glycolipids

They are carbohydrates whose constituent mono, di, tri and tetra saccharides include glucose, mannose, galactose, rhamnose, sulphate and glucuronic acid. Most known biosurfactants are glycolipids. The carbohydrates link with long-chain aliphatic acids or hydroxyaliphatic acids, this combination is achieved either by an ester or ether group. [19] reported that the best known are the rhamnolipids, trehalolipids and sophorolipids in this taxa. Other microbially produced microlipids are cellobiolipids, products of *U. zeae* and *U. maydis*, also Mannosylerythritol lipids from *Candida abartica* [19].

Lipopeptides

Lipopeptides which possess a high surfactant activity such as surface active properties and antibacterial activity are produced by a diverse group of *Bacillus subtilis* (Rashedi et al., 2005). Gramicidin and Polymyxin which are lipopeptides from *Bacillus brevis* and *Bacillus polymyxa* respectively, have striking surface properties. These are composed of a lipid fixed to a polypeptide chain. Surfactin is the most studied, others are lipids, iturin and ornithine [19]. Due to microbial oxidations some fatty acids are produced from n-alkanes, these fatty acids are considered as surfactant. Of the lipopeptide biosurfactants produced by *B. subtilis*, surfactin is one of the most effective.

Phospholipids

They are vital constituents of microbial membranes. The level of phospholipid increases profoundly when some hydrocarbon degrading bacteria or yeast are cultivated on alkane substrates [20]. Phosphatidylethanolamine is a phospholipid which is mainly produced by hexadecane [19].

Polymeric Microbial Surfactants

Emulsan and liposan are the best studied of these group of microbial biosurfactants. Most biosurfactants are polymeric heterosaccharide containing proteins mannoprotein and polysaccharide protein complexes [17].

Particulate Biosurfactant

Hydrocarbons are partitioned by extracellular membrane vesicles giving rise to microemulsion that engages chiefly in alkane uptake by microbial cells. When cultivated on hexadecane, *Acinetobacter* sp builds up extracellular vesicles with a booming density of 1.58g/cm³ and

diameter of 20-50mm. These vesicles seem to be involved in the absorption of alkane by *Acinetobacter* sp. [17].

MECHANISM OF ACTION

Surfactants work in three different ways: Emulsification, Solubilization and Roll-up.

1. Emulsification: The surfactant lowers the oil/solution interfacial tension and makes easy emulsification of the oil.
2. Solubilization: Through interaction with the micelles of a surfactant in a solvent (water), a substance spontaneously dissolves to form a stable and clear solution.
3. Roll-up mechanism: The surfactant lowers the oil/solution and fabric/solution interfacial tensions and in this way lifts the stain off the fabric.

APPLICATIONS OF SURFACTANTS

As a result of its good physicochemical properties, low toxicity and bio degradability, bio surfactants have applications in enhanced oil recovery, crude oil drilling lubricants, health care, and the food processing industry [22]. Surfactants are useful in many ways to our daily lives. They are found in drinks, foods, vehicles, clothes and body-cleaning products. Healthy living and life quality depends on availability and safe use of surfactants.

Improved removal of contaminants

Applying surfactants as mobilizing agents is a way of intensifying the solubility of PAH. Micelles, which increases the solubility of PAH are formed above the critical micelle concentration (CMC), its use in soil restoration technology is therefore plausible [23]. Diffusion path length linking site of absorption and site of bio-uptake by the microorganism is decreased by surfactants which help microbes adsorb to soil particles occupied by pollutants [24].

The use of surfactants to increase desorption and apparent solubility in the aqueous phase is an option to increase bioavailability of PAHs. Aronstein et al., 1991; Laha and Luthy, 1991; Laha and Luthy, 1992 and Scheibenbogen et al, 1994 all reported improved removal of contaminants in lab and field studies by both synthetic and biosurfactants. Many biosurfactants have been applied in remediation of the environment, like soil slurried in bioreactors [25, 26, 27, 28, 29] and accelerating the biodegradation of hydrophobic hydrocarbons in oil-contaminated beach soils [30]. Randhir and Kar, 2003 showed that washing with surfactant solutions is effectual in removing hydrocarbons, chlorinated solvents or polychlorinated biphenyls from soil [31].

Tertiary oil recovery

For over thirty-five years surfactant flood tertiary oil recovery has been in use after waterflooding in depleted oil reservoirs, especially in the USA [32].

Conventional methods for oil recovery have been in three steps; primary depletion, secondary recovery and tertiary recovery. According to Craft et al., 1991, the natural reservoir energy is used to achieve displacement of oil from the porous rocks to the producing wells in primary depletion [33]. This achieves 10-20 percent of the original oil in average. For secondary recovery methods, an injected fluid such as water is used in treating the oil to immiscible displacement. Green and Willhite, 1998, estimated that about thirty to fifty percent of the original oil can be recovered throughout the lifespan of a fully developed reservoir in which primary and secondary methods have been strictly applied [32]. The rest of the oil is entrapped in the permeable media as a result of capillary forces such as surface and interfacial forces. It is also attributed to viscosity forces and reservoir heterogeneities, resulting in low displacement efficacy [32]. Due to the above reasons, lots of enhanced oil recovery (EOR) methods are being evolved and used. EOR methods is reliable for regaining a relevant portion of the remaining oil after traditional methods have been used. Development of many modifications of EOR methods have been going on to regain an

appreciable amount of the remaining oil. Some EOR methods introduce a hot invading phase like steam, hot water or a combustible gas so as to increase the temperature of oil and gas in the reservoir to make their supply easier to the production wells [32]. The EOR technique which we are most concerned about is chemical flooding which consists of alkalis, surfactants and polymers or a mixture of the three. The residual oil can be stimulated by introducing alkali and surfactant agents which reduce interfacial tension (IFT) between oil and water. This systematic treatment has recently gained attention because of exorbitant oil price.

Surfactants create environmental problems

Side effects in the environment and industrial processes can result with the wide range of surfactant applications. Paxeus, 1996 noted that the use of surfactants could also lead to environmental pollution and cause a sequence of difficulties for wastewater treatment plants [34].

Surfactants enhanced industries

Surfactants are of general use in lots of commercial and industrial products and processes worldwide. Surfactants are required greatly for many industrial processes like metal treatments, pesticides, pharmaceutical products, particle growth, oil production and colloid stability, that is apart from its demands in detergents and soaps which we use daily [34 and 35].

Surfactants in foods

The demand for surfactants has grown in the production of a large number of food items ranging from solubilization of oils, liquor emulsification, extraction of cholesterol to others like prevention of component separation and solubilization of essential nutrients. A welcome example is slightly iced foam that is 40-50% air by volume known as ice cream. Generating an emulsion is the first step in ice-cream production. The hot ingredients; milk fats, corn-syrup, solids, stabilizers/emulsifiers, sweeteners, milk solids-no-fat and other dry solids are forced through little orifices under moderate pressure (about 15 to 19 MPa) as a result of the homogenization step. Applications of biosurfactant in foods and dermal/transdermal drug delivery systems was also recognized by [36].

Surfactants in health and personal care products

Use of biosurfactant in cosmetic making and soap formulations is a rapidly developing technology [36]. In addition to their application as fabric softeners in detergents; quaternary ammonium surfactants present an example of cationic surfactants that have powerful germicidal action.

Biosurfactants as clean-up in oil storage tank

More than 90% of hydrocarbon stuck in an emulsified sludge was cleaned up by biosurfactants produced from a proprietary bacterial strain in a field study by [37].

Surfactants in biological systems

Surfactant replacement therapy might be used in treating lung diseases such as meconium aspiration syndrome, neonatal pneumonia and congenital diaphragmatic hernia. Respiratory distress syndrome has been clinically treated with the understanding of the pulmonary surfactant system discovered in 1929. Thin aqueous layer lining the respiratory epithelium of the lungs would cause alveolar collapse at the end of expiration due to its high surface tension resulting when there is a lack of surfactant.

Surfactants in oil removal

With regards to the techniques involved in protection of the environment like oil spills removal, water and soil remediation. Biosurfactants have been successfully used in the removal of crude and model oils from sand columns or contaminated ground during the washing process. Biosurfactant had an effectiveness which was similar with those of synthetic surfactant but much

higher than that of natural plant surfactant (saponin) in removing crude oil according to [38]. Its efficiency was also higher than synthetic Tween 60 [39].

Bai et al., 1997 noted that Biosurfactant displayed efficiency much higher than SDS and Tween 80 in removing hexadecane from sand [40].

ORGANISMS THAT PRODUCE BIOSURFACTANTS AND WHY THEY DO

Varieties of microorganisms produce biosurfactants which are either discharged extracellular or adhered to parts of cells. This happens mostly during development on substrates which are immiscible with water [17] (Desai and Banat, 1997). Biosurfactants perform the role of reducing the surface tension at the interface, thereby enabling microorganisms to grow on water-immiscible substrates. Although the molecular mechanism responsible for uptake of their substrates are still not apparent, biosurfactants make the substrate more readily available for uptake and metabolism [17]. Biosurfactants perform another physical role of antimicrobial effects towards diverse microorganisms. Different surfactants usually inhibit different taxonomy, as a rule.

Furthermore, [17] have shown that biosurfactants are involved in cell adherence which confers on them exceptional safety under hostile environmental conditions and in situations when organisms need to discover new habitat for sustenance.

Ability to produce a metabolite is made possible by an organism's gene, therefore, the genetics of the producer organism is a key factor influencing the produce of biotechnological products.

Available raw materials with optimum medium, culture conditions and efficient recovery processes have all been utilized during the bioindustrial production process nevertheless, the process is still usually dependent on the use of high-producing strains. Production process cannot be profitable and compete effectively pending the time the producer organism yields a high final product [41].

Nitschke and Pastore, 2004 noted that *Bacillus subtilis* produces surfactin while *Pseudomonas aeruginosa* produces glycolipids that act as emulsifiers or surface-active agents [42]. These glycolipids then lower the surface tension of hydrophobic molecules thereby causing them to breakdown [43].

Some enterobacterial are able to produce biosurfactants despite being regarded as pathogenic microorganisms [44]. *Escherichia* spp., *Klebsiella* spp., *Salmonella* spp., *Enterobacter* spp., *Serratia* spp., *Hafnia* spp., *Citrobacter* spp., *Yersinia* spp., *Proteus* spp., *Rhanella* spp., *Providencia* spp., *Morganella* spp., *Shigella* spp., *Edwardsiella* spp., *Ewingella* ssp., *Budvicia* ssp., *Tatumella* ssp., *Erwinia* spp., *Koserella* ssp., *Kluyvera* ssp., *Hoganella* ssp., *Moellenella* ssp., *Leminorella* ssp., *Buttiauxella* ssp., *Pantoea* spp are all bacteria that belong to the family Enterobacteriaceae. They are gram negative microorganisms that are so named because they live in digestive tract of man like saprophytes. In the search for microorganisms with useful metabolites from diverse ecosystems, the finding of some enterobacterial which are capable of producing biosurfactants is distinctive [45]. Species such as *Bacillus licheniformis* strain JF-2, *Bacillus subtilis*, or *Pseudomonas fluorescens* [46] have all been widely reported in biosurfactant production. Kebbouche-Gana et al., 2009 reported the screening of some halotolerant bacteria for biosurfactant production. The two strains were halophiles belonging to genera of the family Halobacteriaceae, *Halovivax* (strain A21) and *Haloarcula* (strain D21) [47]. This, according to them was the first report of biosurfactant production at such a high salt concentration.

Among the *Bacillus* species, *Bacillus subtilis* and *Bacillus licheniformis* have been identified as chief biosurfactant producers [48]. *B. sphaericus* and *B. azotoformans* were also found to be able to produce biosurfactants and this was supported by the work of [49]. According to findings, biosurfactant producers are diverse [50], could be as much as a third of aerobic heterotrophs [51] and are found in varied environments [48]. It was discovered that overproduction of rhamnolipids (a biosurfactant) takes place when there is deficiency in nutrient. Desai and Banat 1997 observed that the most potent yield occurs in a limitation of nitrogen [17]. Manresa et al.

(1991) reported that in the stationary phase of growth, and when nitrogen is no longer available, a build-up of rhamnolipids is observed [52].

With regards to phosphorus deficiency, excess yield of rhamnolipids was also monitored [53]. It is therefore likely that rhamnolipids take part in strengthening relocation to nitrogen and phosphorus-rich niches by way of enhancing microbial motility. The studies of Tremblay et al., (2007) and Verstraeten et al., (2008) supports this [54, 55]. Caiazza et al., (2005) and Tremblay et al., (2007) carried out studies establishing the relationship between the presence of rhamnolipids and swarming motility [54, 56].

When probiotic bacteria exudes biosurfactants in vivo, it can be regarded as a resistant mechanism towards other indigenous strains in the urogenital and gastrointestinal tracts and on medical gadgets, this is so because adherence of biosurfactants to a substratum surface improves its hydrophobicity thereby constraining the microbial adhesion and desorption processes [57]. Reduction of adherence of pathogenic microorganism to glass, silicone rubber, surgical implants and voice prostheses have been reported to be an activity of biosurfactants produced by Lactobacilli, also bacteriocin which possess antibiotic properties are products of bacteria [57]. Thaniyavaran et al., (2003) reported that Bacteriocins are produced by both gram positive and gram negative bacteria and are antimicrobial proteinaceous compounds that inhibit sensitive strains [58].

APPLICATION OF BIOSURFACTANTS IN HEAVY METALS REMOVAL

Different types of synthetic surfactants have been employed in heavy metals removal from wastewater. However, there is an urgent need to substitute these synthetic compounds by natural tensoactives. This has led to researches on the use of surfactants of microbiological nature, the so-called biosurfactants. Biosurfactants as wastewater treatment agent are unique and highly favorable due to their effectiveness, low environmental impact, high specificity, high stability, low toxicity, biodegradability and activity at extreme conditions [59, 60, 61, 62].

With rapid urbanization and industrialization increase, environmental pollution has been a major problem that needs to be solved. More challenging is the indiscriminate disposal of these wastes into the environment with the aquatic system acting as sink [63]. With increasing public awareness on the rise, coupled with firmer environmental legislations being introduced, there is need to develop innovative and low-cost techniques to solve this problem. These low-cost technologies will help clean up both organic and inorganic environmental contaminants [64].

In addition to the various health concerns and negative ecological impacts, environmental pollution has increasingly been linked to waste management, sustainability, and climate change. Research on the applications of biosurfactants in mitigating environmental pollution started over two decades ago, but it seems imperative to shed light on how this ties into the overwhelming and growing topic of sustainability. [65]highlighted the importance of the “reduce, reuse and recycle” concept for waste management due to concerns over the rate of generation of hazardous and non-hazardous wastes, and the inherent cost of its treatment and disposal. The authors emphasize the need for cost-effective biosurfactant production to address these growing concerns.

Application of biosurfactant in heavy metal removal from industrial effluents and contaminated sites

Heavy metal removal from contaminated sites has been a major challenge facing environmentalists because heavy metals unlike organic molecules are non-biodegradable and are persistent in the environment. It is therefore important to employ techniques that involve changing the redox state of a metal to a less persistent and toxic form. Microbes have the ability to interact and alter the properties of a large number of toxic and non toxic metals; hence biological techniques can play a major role in heavy metal removal from contaminated water and soils. This is because metals may be used as:

- electron donors or acceptors in the cellular production of energy in microbes,
- cofactors in intracellular and extracellular enzymatic reactions and
- mobilizers of electrons between organisms in syntrophic relationships [66, 67].

These heavy metals poses no toxic effect in microorganisms and aid basic cellular processes due to the ability of the microorganisms to reduce, oxidize, transport, bind and sequester metals. Hence they can be employed in bioremediation [68, 69, 70].

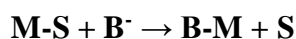
BASIC MECHANISM OF BIOSURFACTANT–METAL INTERACTIONS

From literature, it has been proven that biosurfactant are tolerant to a wide pH range, temperature, salts concentration and are low-cost i.e. they can be produced from readily available renewable materials and high selectivity for a large number of metal ions and organic compounds. The reasons given above have made biosurfactant more effective in heavy metal remediation [71, 72].

The removal of metals from contaminated soils by the action of biosurfactants is in two ways. Firstly, there is formation of complexes between the biosurfactants and the free, non-ionic metals in solution. The complexation speeds up metal removal following Le Chatelier's principle. Secondly, the biosurfactants make direct contact with the adsorbed metal at the solid-solution interface, making the biosurfactants to accumulate at the solid-solution interface [73].

The possibility of formation of biosurfactant-metal complexes is a major factor in their application for the removal of heavy metals from contaminated soils coupled with other driving forces like precipitation-dissolution, ion exchange, counter-ion association and electrostatic interaction [74].

Anionic biosurfactants are able to remove metal ions from contaminated soils due to formation of ionic bonds with metals, giving rise to nonionic complexes that are more stable and stronger than those between the metals and soil.



M-S is the metal contaminated soil, B⁻ is the anionic biosurfactant, B-M is the nonionic metal biosurfactant complex which is more stable and stronger than the M-S complex and S is the soil. After which the biosurfactant-metal complexes are removed from the soil surface. For cationic surfactants, charged metal ions are replaced by them on the surface of the soil particles by competing for some of the negatively charged surfaces of the soil particles.

CONCLUSIONS

Biosurfactants are not as toxic and easily biodegrades than synthetic surfactants. Also, they are extremely potent in varied applications which include oil refining process, and are therefore regarded as very good and potential biotechnological products. Bacteria belonging to the family Enterobacteriaceae may be opportunistic, pathogenic or saprophytic. A few representatives have been isolated and recognized by capacity of producing compounds with biological, chemical and physical properties with prospective advantage for the environment and for humans, like their antimicrobial, emulsifier, chelating, solubilizing capacity etc.

Nevertheless, there still exists lots of studies associated to isolating and identifying novel strains of producers, molecular techniques of synthesis, properties of the biosurfactants produced (physical, chemical or biological) efficacy of the properties, optimization of production and scaling industrially.

Therefore, the questions the scientists keep asking is how to search for a better quality of life, sustainable development and questions that bother round protecting the environment. Use of

biosurfactants instead of synthetic surfactants in numerous sectors permits more prohibitive environmental projections and simultaneously safeguards very good quality.

Though the use of biosurfactant is much safer for the environment, the separation process in getting them from their sources is quite rigorous as the products are available in small amounts. This leads to a higher cost in production than synthetic surfactants. However, as long as researches are channeled in this direction many more organisms that can produce biosurfactants will be discovered and numerous cost-effective ways to separate the biosurfactants will also be invented. Furthermore, in comparison with chemical surfactants biosurfactants are required in little quantities and are efficient under wide range of oil and reservoir conditions.

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