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Review Article

Bacterial nanocellulose, a sustainable alternative, to implement cleaner production in the design of biosensors to detect heavy metals in surface waters

Abstract

While high productivity is positive for growth in developing countries, environmental balance and moderation in pollution levels must be taken into consideration. The dumping of highly harmful industrial waste into river beds, streams, groundwater tables and underground freshwater reservoirs is a clear disadvantage when thinking about sustainable processes. Putting the environment first, we wonder how many thousands of liters of vinase and toxic waste run through our watersheds to end up reusing some bagasse to extract vegetal origin cellulose. It is not about demonizing the product, but rather applying cleaner production technologies to obtain it in a sustainable and ecologically friendly way. The main objective of this review is to propose nanocellulose of bacterial origin as an inert support material for biosensors that detect heavy methals on surface waters. This alternative is sustainable, resistant to temperature and high humidity levels, optical transparency, porous nanostructure and possibilities for surface functionalization. This material has advantages over vegetable cellulose, not only functional, but also from the aforementioned environmental perspective. Heavy metals contamination on surface waters is a global problem. The development of reliable, lightweight and portable biosensors is a necessity for *in situ* detection of the degree of contamination, without the need for cumbersome and often complex sample taking. The performance of a biosensor depends on its ability to immobilize receptors, maintaining their natural activity, against targets in solution, as is the case of our interest. When we propose bacterial nanocellulose as a support it is due to its ability to form covalent bonds and trap by cross-linking. Although due to their high surface area per unit of volume, physical methods are also a possibility that provides versatility of processes that adapt to multiple biosensor formats. Each new discovery of the potential functionalization for bacterial nanocellulose allows us to think of new, more efficient, more environmentally friendly sensors for a multitude of applications. As the contamination of water with heavy metals increases alarmingly due to over-industrialization, it is time to ask ourselves about the cognitive dissonance of using cellulose obtained by traditional means and the aforementioned contamination that they carry to generate sensors to measure the degree of pollution that we generate when producing it.

Keywords: bacterial nanocellulose, biosensors, heavy metals, clean production

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Introduction

Cellulose is a linear polysaccharide consisting of a chain of β (1-4) linked D-glucose units.[1] Although vegetable origin cellulose is widely applied at industrial level, the productive scale generates serious problems on ecosystems where the pulp mills mega industries known as "pasteras" are located.[2] Numerous processes make up the Kraft method, traditionaly employed to obtain microcrystalline grade cellulose. Each of the steps in this method requires large consumption of energy and corrosive and contaminating chemical compounds in even more large volumes. Although the balance of mass and energy of the process is economically beneficial, it results in an environmental imbalance, particularly in underdeveloped countries where the premise of productivity is above caring for the people and the planet.[3] While high productivity is positive for growth in developing countries, environmental balance and moderation in pollution levels must be taken into consideration. The dumping of highly harmful industrial waste into river beds, streams, groundwater tables and underground freshwater reservoirs is a clear disadvantage when science think about sustainable processes.[4] In emerging countries such as Argentina and Uruguay, the location of these "pasteras" generates great concern. A report presented by the University of the Republic of Uruguay (UDELAR) in 2006, summarizes the scientific evidence available on the environmental impacts of the installation of cellulose plants from vegetal origin and the associated forestry model.[5] The report presents analysis and studies of the effects of forestry cultivation on the ecosystem, the benefits provided by natural pastures and the effects generated by liquid effluents at hierarchical levels (molecular, individual, population, community and ecosystem).[5] In our province Tucuman Argentina, we live with one of the oldest paper mills or "pasteras" in the country.[6,7] Concern about the environmental pollution it generates, has reached debates at a legal, political and scientific level.[8] Furthermore, the reuse of waste products from the paper and sugar industries, such as sugarcane bagasse, is an imaginary solution to a huge problem. These types of actions constitute only patches. We wonder how many liters of agrochemicals are poured into the fields, how many towns are fumigated, how many hectares of forest are devastated by monocultures. Putting the environment first, we wonder how many thousands of liters of vinase and toxic waste run through our watersheds to end up reusing some bagasse to extract cellulose. It is not about demonizing the product, but rather applying cleaner production technologies to obtain it in a sustainable and ecologically friendly way. The development of a conscious process, where environmental scientists take a leading role. The breadth of applications that cellulose has at industrial level in all areas and its still undeveloped potential, led scientists around the world to look for sustainable and ecologically friendly options to obtain this raw material with high added value.[9]

Objetive

The main objective of this review is to propose nanocellulose of bacterial origin (BNC) as an inert support material for biosensors that detect heavy methals on surface waters. This alternative is sustainable, resistant to temperature, high levels of humidity, with high optical transparency, porous nanostructure and possibilities for surface functionalization. This material has advantages over vegetable cellulose, not only functional, but also from the aforementioned environmental perspective.

How can we do better?

Although there are proposals about other plant sources of cellulose that involve the reconversion of industrial waste to obtain it. It is the enzymatic, mechanical and chemical treatments of the cellulosic precursors that generate the ecological imbalance. Although they prevent the felling of trees, intensive cultivation for timber production and its consequent damage, waste effluents represent a problematic barrier.[10] In the context of the environmental crisis and climate change that is no longer "imminent" but has become a reality that we are experiencing as a planet, we need a bottom-up approach where cellulose is obtained through biosynthesis using glucose and/or fructose as a precursor through the direct action of specific bacterial strains.[11] Argentina takes relevance in the concern about climate change and its direct relationship with deforestation caused by agribusiness, and presented a series of measures contemplated in the **Climate Change Adaptation and Mitigation Plan**[12] presented in Egypt during COP27.[13–15] This plan proposes environmental impact studies and the criteria that we need to uphold as scientists and as active citizens to protect forests from logging and the action of deforestation on wetlands.[12]

What bacteria can we use for bnc obtention?

Bacterial species such as *Pseudomonas fluorecens*, *Gluconacetobacter xylinus*, *Gluconacetobacter hansenni* produce cellulose as one of their metabolites. *Gluconacetobacter xylinus* is one of the first strains studied as a producer of bacterial nanocellulose (BNC) and has become a model system for the study of the biosynthetic mechanisms of BNC.[16,17] The mechanism of cellulose production by the bacterium *G. xylinus* is the construction of a nanofiber film at the air/culture medium interface.[18–21] BNC is a primary metabolite synthesized inside the bacterial cell that is then twisted into nanofibrils which is mechanically amplified to form microfibrils.[22,23] During the biosynthesis of cellulose chains, they go Van Der Waals forces and hydrogen bonds between hydroxyl groups and oxygen from adjacent molecules promotes the parallel stacking of multiple cellulose chains that form elementary fibrils that then aggregate into larger microfibrils.[24] BNC has distinctive characteristics due mainly to its size and fibrillar arrangement that introduces modifications in its biological and physicochemical properties such as biocompatibility and biodegradability.[25] In addition, the fibers are lighter, have greater optical transparency, its surface is chemically adaptable because allows the annexation of multiple functional groups, improving its mechanical properties.[26] Among the potentialities of BNC are some highly relevant pharmaceutical applications such as biomedical, with the production of implants or scaffolds, translucent films, controlled release systems,[27,28] immobilization of enzymes, tissue engineering.[29] They also exhibit high chemical resistance to dilute acidic and alkaline solutions, organic solvents, proteolytic enzymes and antioxidants.[30,31]

The extensive studies carried out on GRAS bacteria that produce cellulose and the recent discoveries of the possibility of taking advantage of the methods of the cleaner production philosophy, lead us to optimize the production of bacterial cellulose, on a nanometric scale.[31,32] This reality of the process opens up an even wider plethora of possibilities for the applications of a product already widely used in the industry and with a new origin. These are ecologically friendly, sustainable processes from the beginning of production and with 0 waste, since the waste components are reused, adding value through the creation of new products.

Biosensors as devices to detect heavy metals in surface waters

A biosensor is an instrument for measuring biological or chemical parameters. It combines components of biological and physical-chemical nature. It is made up of three parts: the biological sensor can be a tissue, a culture of microorganisms, enzymes, antibodies, chains of nucleic acids trapped in an inert support;[33] the detector can be optical, piezoelectric, thermal, magnetic, among others; the transducer couples the other two elements and translates the signal emitted by the sensor. Heavy metals contamination on surface waters is a global problem. The development of reliable, lightweight and portable biosensors is a necessity for *in situ* detection of the degree of contamination, without the need for cumbersome and often complex sample taking.[34] Biosensors use specific molecules as elements recognition that meet the premises of high specificity and the possibility of remaining inert for long periods of time to extend the useful life of the biosensors.[35]

Why is it necessary to trap biosensor enzymes in inert materials?[36, 37]

In the case of enzymatic biosensors in general, immovilization is necessary to increase the performance and durability of the biosensor by increasing thermal stability, decreasing degradation against pH and increasing solvent stability, recyclability and storage.[33,34] The performance of a biosensor depends on its ability to immobilize receptors, maintaining their natural activity, against targets in solution, as is the case of our interest. It is also related to non-specific adsorption to the solid support.[38–40] Inmobilization techniques by physical methods are: adsorption, entrapment or encapsulation. Encapsulation techniques by chemical methods are the formation of covalent bonds and cross-linking. When we propose BNC as a support it is due to its ability to form covalent bonds and trap by cross-linking. Although due to their high surface area per unit of volume, physical methods are also a possibility that provides versatility of processes that adapt to multiple biosensor formats. Determine which of the techniques generate improvement in the characteristics of a biosensor allow making decisions regarding the process of manufacturing, for example, Shahar et al. 2019 fabricated a microsphere holder from a polymer, this was modified by functional groups that attributed improvements in inmobilization separately in the adsorption and covalent bonding method.[41,42]

Noble metal nanoparticles as sensors of metal ions in water

Within the new universe represented by particles on a nanotechnological scale, new possibilities arise that amplify the sensitivity of existing sensors, and the design of other types of sensors.[43] Their simple synthesis, high surface area and physicochemical malleability make nanoparticles one of the most promising approaches.[44] The fact that the immobilization is reversible or irreversible implies that the biological material may or may not be separated from the support matrix.[45] The possibility of removing the

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biosensor to recover the material makes the separable support matrices an economic and environmental advantage due to the possibility of reusing the materials.[46,47] Flexible plasmonic substrates facilitate the reading of the plasmon wavelength, or dispersion, as a function of the chemical change that the surface of the particles undergoes.[48,49] The concept of "flexible plasmonic substrates" refers to plasmonic nanoparticles (metals such as Au and Ag) impregnated into flexible solid substrates such as bacterial nanocellulose.[50–52] Which presents advantages over conventional rigid substrates in terms of cost, processability and sustainability.[53] Flexible substrates can be used, for example as packaging, can be wrapped around non-flat substrates, or as swabs to collect wound samples.[54,55] In the future, it is expected that flexible plasmonics can be combined with new generation electronics with various functions in the same device.[56] The possibility of using NBC reduces costs and includes improvements, such as its optical transparency compared to the cellulose fibers of conventional paper, also used as a support, improving the efficiency of the sensor.[57] Other flexible substrate that are used to impregnate them with low-cost plasmonic nanoparticles are: filter paper, nanofibers, elastomers, plastics, carbon nanotubes and graphene. Organic material has been used for the synthesis and stabilization of nanoparticles simultaneously, taking advantage of the structure of natural polymers such as cellulose and gum arabic, where the hydroxyl groups and oxygens not only hold metal ions in their structure by interactions ion-dipole, but also stabilize the nanoparticles due to the strong interactions of these with the surface atoms, this has been carried out using chemical reductants.

Bacterial nanocellulose advantages

Nanocellulose in its native form, that is, of plant origin, presents limiting barriers in its applicability as a chemical sensor. BNC compounds expand the concept of versatility and sustainability, being of great interest as supports in the design of chemical sensors. Its surface functionalization possibilities open up a plethora of possibilities with respect to other inert supports.Transforming into a nanostructured, porous polymer that receives chemical groups, modifying its specificity according to the functional need of the biosensor. It is anticipated that the surface possessing electrical properties will be an excellent sensing mediator. The electrostatic interactions found between the different charges found in nanocellulose also represent an advantage. Compounds and analytes play an important role in manufacturing ion exchange and permselective membranes. These developed from BNC compounds can be modified by changing the functionality of its surface and its selective permeability properties for new sustainable, biodegradable biosensors design.

Conclution

Studies on the functionalization of bacterial nanocellulose with other nanomaterials useful for the early detection of pollution in surface waters are still in their first stages. There are several other types of nanomaterials that would potentially behave as good conductors capable of improving and expanding the properties of bacterial nanocellulose compounds used in heavy metal and agrochemical detection applications. This is a still incipient area of research. But each new discovery of the functionalization potential of bacterial nanocellulose allows us to think of new, more efficient, more environmentally friendly sensors for a multitude of applications. As the contamination of water with heavy metals increases alarmingly due to over-industrialization, it is time to ask ourselves about the cognitive dissonance of using cellulose obtained by traditional means and the aforementioned contamination that they carry to generate sensors to measure the degree of pollution that we generate when producing it.

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Conflicts of interest

Authors declare there is no conflict of interest.

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