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Bioremediation: Perspectives of the use biopolymers systems for slow release nutrients

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Abstract: Biostimulation (microbial stimulation) is the most effective bioremediation strategy, thus it's widely used in Brazil, and is highlighted for its simplicity and low cost. In biostimulation, polymeric systems are used in controlled release of nutrients (SRN) in order to maintain their adequate concentration to stimulate xenobiotic molecule-degrading microorganisms. The use of biopolymers systems ensures biodegradability, low cost and low toxicity over synthetic ones. Despite advances in studies with naturally occurring polymers, few are used as SRN for bioremediation applications. Thus, there are still remaining gaps to be filled concerning release efficiency, and effects on microbial growth and degradation of xenobiotics. Therefore, this work aims to explore the results and advances of these biopolymeric systems used in SRN and their future perspectives in bioremediation.

Keywords: Data analysis, Statistical analysis, Bioremediation, Biopolymer, Slow release systems, Microbial activity, xenobiotic compounds.

Adherence to the BJEDIS' scope: This work is closely related to the scope of BJEDIS as it presents a data analysis that allows foreseeing trends on slow release systems based on biopolymers.

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1. INTRODUCTION

Anthropogenic action in nature, mainly from industrial activities, is today the primary cause of increased global contamination. The contamination mechanism can occur by diffusion in low quantities over long periods of time or by occasional accidents, in high amounts. Although the latter is more noticeable, the minimum daily discharges are more difficult to contain and, therefore, the most worrying [1]. Researches of contamination in Europe showed that, by 2014, more than half of the pollution was from organic pollutants, especially aromatic and chlorinated compounds. [2–4]. Both of these compounds are classified as Persistent Organic Pollutants (POPs), which are stable compounds capable of remaining in the environment for long period and are characterized by high toxicity, teratogenic, mutagenic and / or carcinogenic potential [5; 6].

Much research has been done in the investigation of its capacity to degrade organic compounds through techniques such as photocatalysis, sonocatalysis, ozonation process, microbial activity, etc [7–9]. Recent studies in microbial degradation technique, also referred as bioremediation, have shown the enormous potential of the technique in the remediation of contaminants such as organic compounds, for instance, HPA´s and organochlorines, and heavy metals [10–14]. More than a half percent of HPA´s mass can be degraded by microbial community [15]. It was even used in one of the world’s biggest oil spill accidents that took place in Alaska (Exxon Valdez, 1989) [16].

The bioremediation can be used *in situ*, in both marine and soil environments which provides a lower cost and lower secondary contamination [17]. However, nutritional deficiency in the medium can impair the stimulation and growth of these microorganisms. Thus, polymeric materials are studied for slow release of these nutrients in order to keep the microbiota active in the medium. Despite presenting more predictable physicochemical characteristics, the high cost and environmental problems related to petrochemical polymers guide the way to more sustainable studies using biopolymers as an alternative coating [18–21].

Therefore, this work aims to explore the results and advances of these biopolymeric systems used in SRN and the future perspectives of these materials in bioremediation.

1.1. Bioremediation Technique: Evolution and trends

Although one of the earliest studies of microbial degradation found in the literature dates back to the 1920s, where author reports the ability of microorganisms to utilize certain organic compounds as a source of carbon and energy for cell construction, the term bioremediation was introduced only around the decade of 1940 to refer to the application of different strategies to make the microbial degradation process more effective [22; 23]. Therefore, it is still a technique with relatively few studies that are in vertiginous growth.

A survey carried out in platform magazines such as Periodic Capes, Wiley, and Springer Link, in March 2020, with the keyword “bioremediation,” evaluated the significant advance in the number of research related to the topic. The search engine encompassed all types of publications. It was only from the 1980s that research on the topic began to emerge. The most significant jump occurred between the 90s (160 publications) and the 21st century (6849 publications), with a growth in publications greater than 4000% (ex. Periodic Capes). However, the number of searches continues to grow exponentially every decade. This recent advance reflects the search for less harmful remediation mechanisms to the environment taking advantage of techniques capable of stimulating the degradation of pollutants by biological agents at a reduced cost.

Each of the tested bibliographic bases presented different numerical results. These different numbers result from the size of the collection made available and the own metrics used by each of the databases. Despite different numerical values, they all followed a growth profile. The data collected in the research were adjusted with the aid of two different models. The first model was the linear model and the second model was the quadratic model. The modeling was carried out between 1990 and 2020, a period in which all counts were greater than 1. Figure 1 shows the evolution of the number of publications in the studied period and the models tested. The model parameters are shown in Table 1.

The calculated R2 data shows that the quadratic models produced a much better fit than the linear models. Also, the RMSE values of the quadratic models are substantially lower than those obtained for the linear models, proving that the quadratic ones are more suitable for the interpretation of the numerical data extracted from the researched databases.

The best fits obtained from the quadratic models indicate a strong tendency to accelerate the number of studies involving bioremediation over time. These data were extrapolated to the year 2030. The numerical results obtained via extrapolation are equal to 105318, 35190, and 20228 for the Capes, Springer, and Wiley bases, respectively. When the figures extrapolated for the year 2030 are compared to the figures collected in 2020, the results point to extrapolated growths equal to 89%, 89%, and 86%, respectively. These values prove that the collections reflect the number of studies developed by the scientific community regardless of its preferred base. Thus, the strong growth in research related to the subject of bioremediation is a trend.

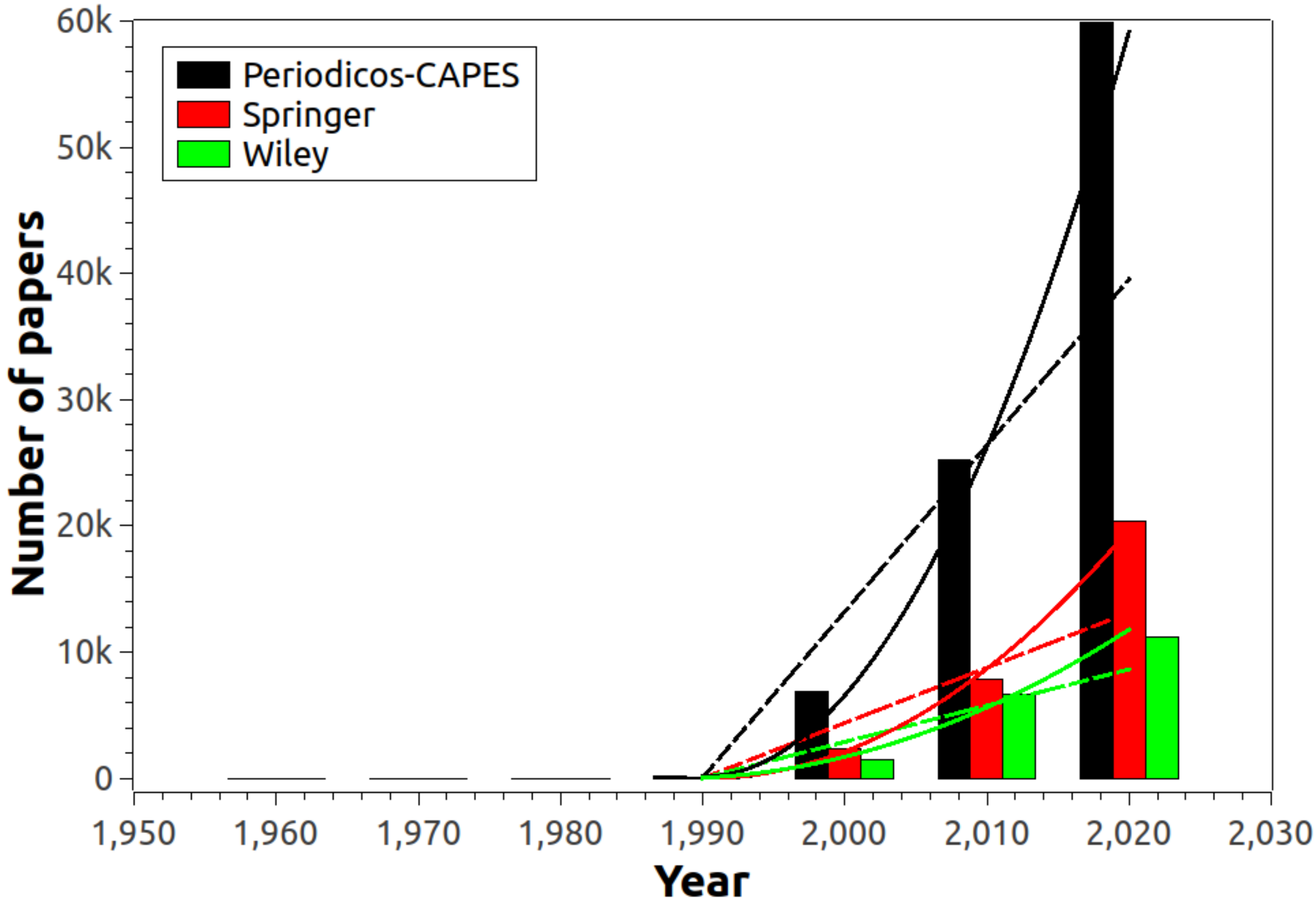

Figure 1. Publications on "bioremediation" (March 2020). Linear models (**---**) and quadratic models (**—**).

Table 1. Linear (Lin) and quadratic (Qd) regression models for the number of publications.

Source	Y-intercept (a0)	Slope (a1)	Quadratic coefficient (a2)	R^2	Adjusted R^2	RMSE
CAPES-Lin	$-(2.619\pm0.009)x10^{66}$	$(1.317\pm0.005)x10^3$	-	0.9961	0.9883	80.5673
Springer-Lin	$-(8.709\pm0.054)x10^5$	$(4.377\pm0.027)x10^2$	-	0.9974	0.9921	46.8824
Wiley-Lin	$-(5.717\pm0.044)x10^5$	$(2.873\pm0.022)x10^2$	-	0.9852	0.9857	32.3753
CAPES-Qd	$(2.623\pm0.023)x10^8$	$-(2.636\pm0.023)x10^5$	(6.623±0.058)x10	0.9999	-	7.7080
Springer-Qd	$(8.831\pm0.136)x10^7$	$-(8.874\pm0.137)x10^4$	(2.229±0.034)x10	0.9999	-	11.4179
Wiley-Qd	$(4.458\pm0.104)x10^7$	$-(4.486\pm0.104)x10^4$	(1.128±0.026)x10	0.9995	-	15.3850

Biostimulation of microorganisms is the most effective bioremediation strategy, it's the most used technique in Brazil, and is highlighted for its simplicity and low cost [24–26]. The biostimulation's primary objective is to stimulate the development of the native soil microbial population by adjusting the environmental parameters [17; 27–34], such as aeration, soil humidity, pH, etc., although the nutritional correction is one of the main limiting parameters of the process [35; 36]. In nature, nutrients, mainly nitrogen, can be found in low concentrations or even be unavailable in the contaminated environment and tend to reduce further during the process of contaminant degradation affecting microbial growth and, consequently, the conversion of the contaminant [24]. The solubility of these nutrients, mainly in the form of inorganic salts, and the associated leaching and maintenance of the availability of said nutrients leads to a substantial economic disadvantage, along with the possibility of increased toxicity and eutrophication of the soil [37; 38]. Specifically to urea, the environment does not assimilate 70% of the nutrient. Besides that, losses also occur through ammonia volatilization [39]. In order to minimize the dissolution of the urea and maintain a more regular release profile, polymer-based coatings have been studied. The incorporation of the nutrient may vary according to the polymers used [18; 40]. Nutrients can be dispersed in the matrix or encapsulated within the polymer in core-shell morphology [41]. Thus, the release mechanism is influenced by the diffusion and degradation process of the polymer [19; 42].

1.2. Common biopolymers used in SRN

A wide variety of polymers made from natural origins have been studied aiming to act as barrier materials decreasing the nutrient delivery speed [91, 92[45–94]]. However, the gradual delivery of the asset to the medium depends on some factors such as the type of polymeric coating, the chemical structure and the thickness of the biopolymer, for example. The hydrophilicity of most of the studied biopolymers is one of the factors that most hinder the use of these materials in SRN systems. Studies focus on modifications of these materials to acquire a hydrophilic/hydrophobic balance in the structure through reactions such as graftization, crosslinking, etc [21, 95].

Some of the main biopolymers studied are starch (see Fig 2(a)), chitosan, cellulose (see Fig 2(b)), and lignin. Despite the diversity, starch stands out as one of the most used biopolymers due to its low cost, high availability, and good biodegradability. However, it is worth highlighting the importance of modifications in its structure to improve the hydrophilic and mechanical properties [96].

In [97], the concentration of N, N - methylene-bisacrylamide was evaluated as a crosslinking agent in the structure and release profile of the starch-based system. The higher content of the crosslinker allowed an increase in porosity and a reduction in pore size, therefore, controlling the permeation of water and nutrients out of the system. In [98], was studied the coating thickness of modified starch with polyvinyl alcohol (PVA) and citric acid, as a crosslinking agent, in urea release and its diffusion rate. The release of 100% of urea with different coating thicknesses was measured by UV-Vis absorbance, during a defined period. The authors observed that although the nutrients release time increases with increasing thickness, large pores and defects in coverage, such as the absence of uniformity of thickness, limited the release system.

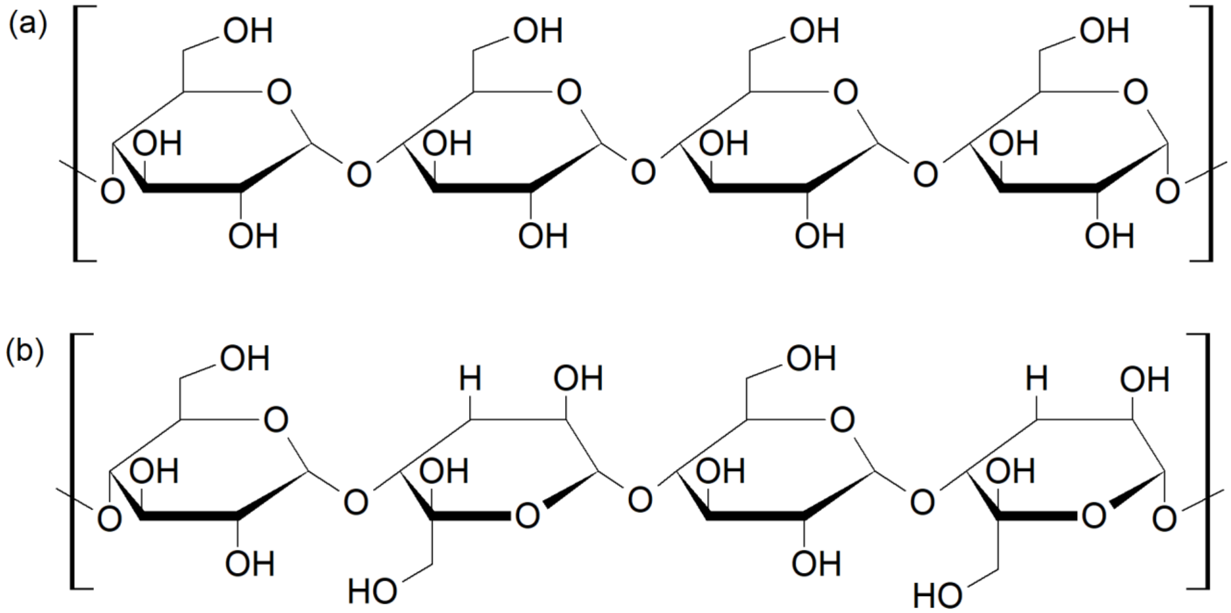

Figure 2. Chemical structure of starch (a) and cellulose (b)

Despite the benefits, introducing a material into the environment requires care: the toxicity of the material must be extinguished and its biodegradability understods. The best of these materials cannot cause environmental damage and must be utterly biodegraded after the full release of the active agent [99]. The degradative capacity of natural polymers is much higher than other synthetic polymers. However, it is possible to improve this biodegradability by modifying the system [100; 101]. For instance, the influence of palm fibers increased the biodegradability of the poly (lactic acid) sample (PLA) by 29% [102]. The high hydrophilicity and low adhesion of the fiber to the polymer matrix promoted a rupture along with the composite structures that facilitated the process of degradation of the material [103]. In PLA, the degradation is speeded up due to the hydrolysis. The same occurs with other polyesters. The hydrolytic degradation mechanism occurs with the diffusion of water into the polymer, promoting the breakage of the ester bonds, as shown in Figure 3 [104].

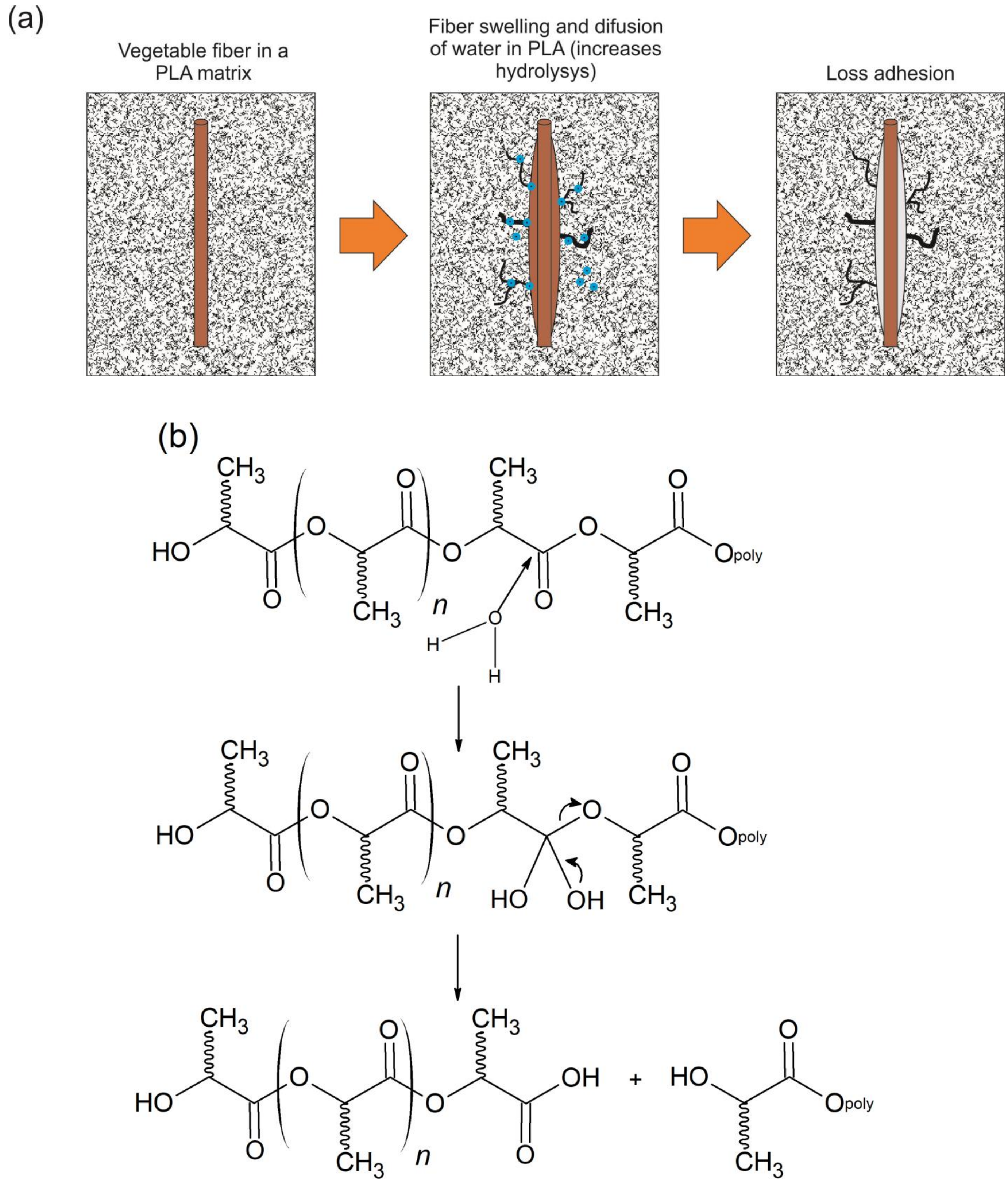

Figure 3. Scheme of percolation of the water until the loss of adhesion between the fiber and the polymer matrix (left). Mechanism of hydrolysis of PLA in the presence of water (right).

Plant fibers present several macromolecular components. Among them, the most interesting for the slow release applications are cellulose and lignin [105–111].

Lignin is a highly branched macromolecule responsible for fiber protection, including against humidity [112]. This macromolecule is composed of three types of phenylpropane units, joined by carbon-carbon bonds and ester bonds, arranged in a complex, highly branched three-dimensional structure, as shown in Figure 4 [113]. The use of lignin in systems for the slow release of nutrients is quite attractive. However, there may be variations in the hydrophobicity of this material, depending on the method used for its extraction. Regardless of the method, the acetylation of lignin promotes a better release of the active substance [98; 114].

Cellulose is the major component of fibers of plant origin. Its chemical structure is composed of carbon, hydrogen, and oxygen atoms that form polysaccharides $(C_6H_{10}O_5)_n$ of 1,4-β bonds [110]. It is a highly crystalline polymer due to the straightness of its chairs and their intermolecular hydrogen bonds. Its crystallinity provides a high mechanical resistance in the fibers and best supports the effects of degradation, whether thermal, mechanical, or chemical [107]. As a coating material, it allows greater hydrophobic control of the system and, consequently, delays the release of nutrients into the environment. Good results are also achieved by modifying cellulose. Authors who have worked with modified cellulose have observed 35% higher nutrient retention than the uncoated system [19; 110].

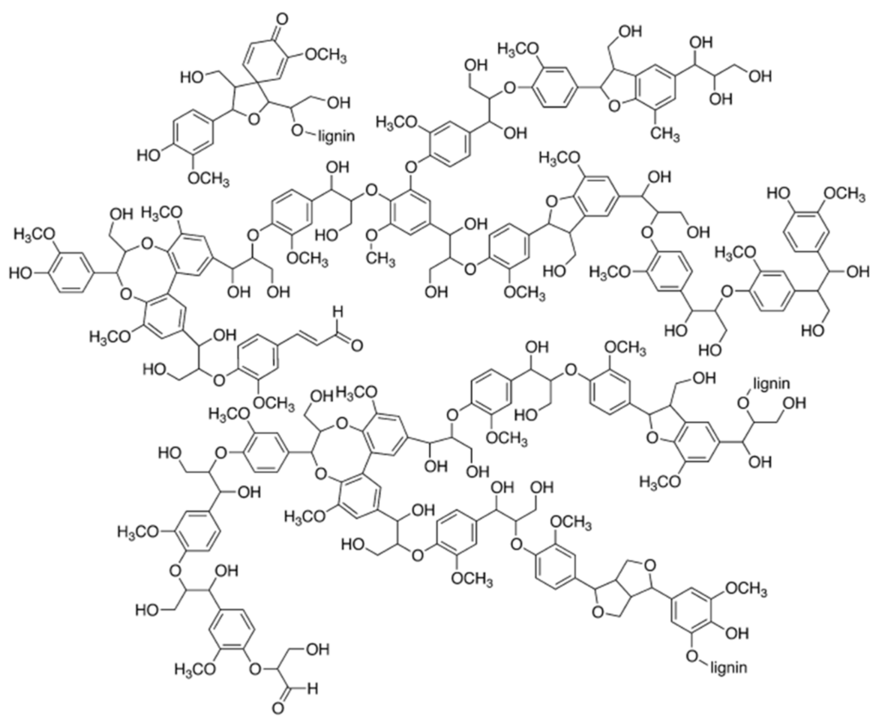

Figure 4. Partial chemical structure of the macromolecule lignin [113].

In addition to crosslinking and chemical modification, the use of double-coating systems may be a strategy to improve the nutrient release profile [115]. In a double coat system with crosslinked starch, as an outer layer and ethylcellulose (EC), as the inner layer, the modified cellulose provided to the system a better balance on the hydrophilic and biodegradable properties of the starch [19]. Another case of double coating is diatomite (outer layer), fossil material formed by the deposition of algae, and xanthan gum reticulated with epichlorohydrin (inner layer), in which the higher crosslinking density of the material allowed the better release of urea after 28 days (79%) [116].

Superabsorbent polymers (SAP's) are hydrogels able to absorbing and retaining large amounts of water. Superabsorbent polymers based on starch or chitosan have been widely studied as nutrient encapsulants for slow release [97; 117–119]. For this application, the type of crosslinker and its content play a crucial role in the rate and profile of the fertilizer release from the SAP structure. Surprisingly, the higher content of the crosslinker in the starch structure produces elevated porosity, followed by the reduction of the size of the pores, modifying the permeation of water and nutrients to the environment [120].

Despite evident lack of articles that study nutrient release systems based on biopolymers with a focus on bioremediation, good outcomes can and should stimulate new research.

Alginates have excellent coating properties. The reaction with polyvalent cations creates cross-linked structures. These structures produce resistant polymer gels or insoluble polymers [121; 122]. The high coating of this material, when associated with commercial starch, was responsible for higher cell growth than in the uncapped urea case. This growth of microbial mass was responsible for the degradation of 43.6% of the hydrocarbons present after 10 hours of the experiment [122]. Other works with sodium alginate have been proposed with the addition of bentonite for release purposes. The improvement in porosity and viscosity are some of the advantages of clay in improving the release of compounds [121].

The structure of the polymer and its sorption capacity may also contribute to the degradation of the xenobiotic since it can increase the bioavailability of the contaminant to the microbial biomass. The term bioavailability is associated with the presence of a vast area of contact between the contaminant and the microbial agents [123; 124]. In the case of chitosan as a coating material, this biopolymer favored the bioavailability of polycyclic aromatic hydrocarbons (HPA's) with four or more aromatic rings. Besides, the degradation of chitosan, rich in nitrogen, represented a secondary source in the nutrient supply [124]. Reticulated chitosan was filled with modified cellulose, producing good release results. These materials reached 75% of release after the 30th day [125]. However, in the acidic soil, the release of the nutrient may be accelerated due to the dissolution of the amine groups of chitosan [126].

The wide variety of biopolymers and the combination of these materials to create new environmentally correct systems with better properties make these slow-release systems promising for commercial applications, not only in agriculture but also in bioremediation. With that in mind, over the past few years, more and more patents have been applied for reaching greater nutrient-retaining capabilities [127–130]. The variety of biopolymeric matrices studied, and their modifications, as well as the different structures of the system, have achieved satisfactory responses and can be used according to the particularities of each application site.

2. CONCLUSION AND FUTURE PERSPECTIVES

Although still recent and in development, some of these biopolymer systems are already showing promising results. It occurs due to the combination of the properties of the coating with the environmental advantages of the biopolymers. However, in order to guarantee the controlled release of the active substance, are required further studies on the characteristics of biopolymers, such as biodegradation, hydrophobic/hydrophilic balance and possibilities of modifications (crosslinking, graphitizing, composite or two-layer coating) thickness and uniformity of the coating and the form of incorporation of the active substance.

Despite advances in biopolymeric coating release studies, few of them are directed at the bioremediation technique. The majority of applications are focused on the nutritional improvement of the soil for the agricultural industry. Thus, there is a need for further studies evaluating new biopolymers, their rate of nutrient release, as well as the effects on microbial growth and the rate of degradation of xenobiotics.

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CONFLICT OF INTEREST

None.

Sample CRediT author statement

Fernanda V. de Carvalho: Conceptualization, Methodology, and Writing-Original draft preparation. **Luisa V. da Silva**: Methodology and Writing. **Thuanny de A. Moraes:** Methodology and Writing. **Fernando G. de Souza Junior:** Conceptualization and Supervision. **Selma G. F. Leite:** Conceptualization. **Ivonete O. Barcellos:** Reviewing and Editing. **Eldho Elias:** Statistical studies. **Sabu Thomas:** Editing. **Kaushik Pal1:** Reviewing. **Thinakaran Narayanan:** Data analysis.

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