Journal Pre-proofs

Review

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PII: S0960-8524(22)00247-4
DOI: https://doi.org/10.1016/j.biortech.2022.126918
Reference: BITE 126918

To appear in: *Bioresource Technology*

Received Date: 17 January 2022
Revised Date: 23 February 2022
Accepted Date: 24 February 2022

Please cite this article as: Dzionek, A., Wojcieszyńska, D., Guzik, U., Use of xanthan gum for whole cell immobilization and its impact in bioremediation - A review, *Bioresource Technology* (2022), doi: https://doi.org/10.1016/j.biortech.2022.126918

Use of xanthan gum for whole cell immobilization and its impact in bioremediation - A review

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ABSTRACT

Xanthan gum is one of the exo-polysaccharides produced by bacteria and is characterized by unique non-Newtonian properties. Its structure and conformation strongly depend on the fermentation conditions and such factors as temperature and ions concentration. The properties of the xanthan gum were appreciated in the controlled drug delivery but in the crosslinked form. Due to its ability to enhance the survival rate of immobilized bacteria, the potential of a crosslinked form is promising. Unfortunately, xanthan gum crosslinking procedures often require toxic substances or harsh environmental conditions, which cannot be used in the entrapment of living cells. In this study, we summarised a crosslinking method that could potentially be modified to reduce its toxicity to living cells. Moreover, this review also includes using xanthan gum in bioremediation studies and possible utilization methods to avoid carrier accumulation in the environment.

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KEYWORDS

Xanthan gum; immobilization; bioaugmentation; microorganisms; crosslinking; entrapment.

1. INTRODUCTION

We face a global pollution problem in the age of extreme industrialization and civilizational development. However, the environment responded with an enormous diversity of microorganisms that developed various enzymatic systems. With the advancement of science, scientists nowadays can understand and describe more complicated metabolic pathways for pollutant breakdown found in bacteria and fungi (Thelusmond et al., 2018; Tian et al., 2020; Olivera et al., 2019; Li et al., 2018). Based on this knowledge, introducing microorganisms with specific enzymatic systems into a contaminated area or bioremediation system can improve pollutant breakdown when indigenous microflora cannot do it (Wang et al., 2020; Dzionek et al., 2016).). However, the introduction of foreign strains without any carrier into already formed and stabilized microflora can significantly affect the viability and performance of introduced cells.

One of the ways to increase the survival chance of bioaugmented strains is their immobilization (Dzionek et al., 2016; Ahmad et al., 2020). The most common method of immobilization in bioremediation is adsorption on the carrier surface and is based on the natural ability of microorganisms to form a biofilm. However, strains that cannot form a biofilm or are GMO cannot be immobilized by this method. Additionally, antibiotics, toxic metabolites, or competition in bioremediation systems, especially in wastewater treatment plants, often influence formed biofilm (Dzionek et al., 2020). To resolve this issue,

microorganisms could be trapped in polymers. By this method, cells leaking into the surrounding medium are significantly limited, and most importantly, they are separated from indigenous microflora. Additionally, by choosing the suitable adsorbing polymer, the contaminant could be delivered in portions to the cells, and as an effect, higher concentrations of the compound are degraded (Berillo et al., 2021).

Due to the specification of wastewaters, which contain a high concentration of ions, metabolites, and solvents, not all polymers are suitable for use. Alginate or κ-carrageenan beads often dissolve in the first days of incubation as an effect of ions replacement in their structure (Cruz et al., 2013). On the other hand, most of the stable in wastewater polymers used for microbial cells entrapment require harsh crosslinking procedures, resulting in a low survival rate of trapped cells. For example, to perform crosslinking of poly(vinyl alcohol), boric acid needs to be used. Obtaining acrylamide hydrogel with trapped bacterial cells requires exposure to gamma radiation (Polakovič et al., 2017; Singh et al. 2019; Jiang et al., 2021; Berillo et al., 2021). Because of that, there is a need to develop new entrapment methods, which will be non-toxic for cells and provide a carrier with high chemical and mechanical stability.

Many scientific research groups have recently been interested in biopolymers due to their biocompatibility and natural origin. Especially, polysaccharides have created extensive interest as carriers. One of them, xanthan gum, is a well-known additive in the food and cosmetics industry. The growing popularity of this gum is a result of its unique non-Newtonian behavior in aqueous solutions. Even at a very low concentration, it acts as an efficient thickener, which helps with mixing, filling, pumping, and pouring the products. Additionally, unlike other gums, it has high stability in a wide range of temperature (up to

90^0C), pH (2–11), and ionic strength (up to 150 g/L NaCl). Moreover, xanthan gum improves the emulsion colloidal stability and reduces its vulnerability to shear forces during processing and packaging. It was observed that it could form high viscosity solutions at low shear forces. Simultaneously, its viscosity decreases with increasing shear rate. Such positive effects make xanthan a perfect emulsion stabilizer in sauces, salad dressing, and face or body creams. It could compete with other natural gums due to the chemical reproducibility, relatively easy production, and high efficiency after using small amounts (0.05 - 0.50%) (Rosalam et al., 2006; Garcıa-Ochoa et al., 2000).

Despite the xanthan gum's excellent features in the non-bonded form, its crosslinking methods have been developed. Xanthan is also able to form physical hydrogels. However, they are not as resistant as chemically crosslinked. Most of the xanthan gum crosslinking methods were designed for the controlled drug delivery or implantology to obtain a biologically inert material, which is also very durable in changing and harsh environmental conditions. Unfortunately, none of these protocols considers the presence of sensitive living cells. The influence of cross-linkers on the final parameters of xanthan hydrogels, such as chemical and mechanical strength, shape-memory effect, and swelling, have been extensively investigated (Izawa et al., 2010; Tao et al., 2016; Simões et al., 2020). Based on the conditions and factors that occur in the bioremediation studies, especially in the wastewater treatment plants, a carrier with these properties would be desirable. However, using a crosslinked xanthan gum for the entrapment of living cells requires significant changes in the preparation protocols to minimize the negative impact of toxic reagents or harsh environmental conditions on trapped cells. According to the

literature, living microorganism incorporation in crosslinked xanthan gum was performed only once (Dzionek et al., 2020).

This review aimed to take a closer look into a xanthan gum structure and properties to discuss its recent crosslinking procedures, which could be potentially modified for trapping living cells. Besides, the recent xanthan gum applications in immobilization for bioremediation and the possible utilization methods were precisely reviewed.

2. ORIGIN

One of the adaptation strategies of bacteria against harsh environmental conditions is biofilm production. This feature is an essential aggregation method in which a gel-like matrix surrounds microbial cells. Biofilm is build up in 95% of water, and microorganisms, and extracellular polymeric substances (EPS). The EPS composition mainly depends on the conditions under which they are formed, but typically it constitutes polysaccharides, proteins, and nucleic acid. One of the major roles of the EPS in the biofilm physical and chemical properties is the water holding abilities assured by polysaccharides. The most known polysaccharide present in the biofilm is alginate, which can be isolated from brown algae and bacteria like *Pseudomonas aeruginosa* or *Azetobacter vinelandii* (Dzionek et al., 2016; Bergmann et al., 2008). However, another example of biofilm's polysaccharide is commercially available xanthan gum.

Xanthan gum is a fermentation product of the Gram-negative bacteria from the *Xanthomonas* genus. All of the bacteria from this genus are known as plant pathogens. Most of the commercially available xanthan gum, however, is produced by the strain *Xanthomonas campestris*. These cells occur as single straight rods and are motile due to a

single polar flagellum. Additionally, they are obligate aerobe, chemiorganotrophic, and create yellow, smooth, and dense colonies. They oxidize glucose in the Entner-Doudoroff pathway (predominantly) or in the pentose phosphate pathway (only 8 – 16% of the total consumed glucose). It was observed that the xanthan gum is produced in the capsules (Garcıa-Ochoa et al., 2000).

The European List of Permitted Food Additives classified xanthan gum as E415 and recognized it as a non-toxic additive for humans. Moreover, the US FDA has been given the GRAS status (Generally Recognized as Safe) to an ethanol precipitate of xanthan gum (Petri et al., 2015; Gils et al., 2009). Its safety properties against food and pharmaceutical applications have been extensively studied. Additionally, xanthan gum does not influence growth and does not cause eye or skin irritation (Garcıa-Ochoa et al., 2000).

3. CHEMISTRY

3.1. STRUCTURE

Xanthan gum is a complexed exo-polysaccharide (Figure 1). Its backbone is made of the repeated units of cellobiose. Due to the xanthan gum's branched nature, side-chains contain a trisaccharide (β-1,4-D-mannose, β-1,2-D-glucuronic acid, α-D-mannose) connected with the backbone (glucose residues) *via* α-1,3 linkages. Additionally, part of the terminal D-mannoses are linked to the ketone group of pyruvic acid. On the other hand, coupled with the backbone, α-D-mannoses are also connected with the acetyl group. Some of the external mannoses contain a second acetyl substituent. As a result, xanthan gum possesses many functional groups such as carboxylic and hydroxyl groups. These groups

can be modified or functionalized to enhance physicochemical properties. It was reported that different production parameters like a bioreactor type (continuous or batch operation), nutrients concentration, pH, temperature, and oxygen transfer rate have a significant influence on the fermentation yield and the chemical structure of the xanthan gum. Moreover, xanthan gum's molecular weight varies from 1 x 10^6 to 20 x 10^6 mol/g, but the average molecular weight is approximately 2 x 10^6 mol/g. These molecular weight differences strongly depend on the association between chains or formed aggregates, which results from the differences in the chemical composition (Garcıa-Ochoa et al., 2000; Rosalam et al., 2006; Petri et al., 2015; Elella et al., 2019).

For example, rhamnose has been identified in xanthan gum produced by pathovar *juglandis* and *manihotis* 1182 (Lawson et al., 1977; Silva et al., 2009). Strain *X. campestris* pv *campestris* 8396 produced gum without a glucuronic acid (Heyraud et al., 1998). Pyruvate content can also vary from almost zero to 8% by changing the medium and bacterial strain (Garcıa-Ochoa et al., 2000; Kurbanoglu and Kurbanoglu, 2007). It was observed that the most crucial feature to change the pyruvate content was the nitrogen source. However, there are different results about the influence of nitrogen on pyruvilation degree. Kennedy et al. (1982) observed higher pyruvate content with increasing concentration of the nitrogen source. In another study, Davidson (1978) reported that limiting nitrogen sources results in less acetate and more pyruvate contents. At this point, it should be noted that too high a nitrogen concentration can also negatively affect cell growth. Since xanthan production is very limited in the exponential phase and is most efficient in the stationary phase (Habibi et al., 2017), changing the nitrogen concentration should be considered carefully only in the stationary phase. Acetate content could be

altered by agitation speed and the incubation temperature. Casas et al. (1999) reported that the maximum acetate ratio was obtained at 500 rpm, and simultaneously pyruvate content remained unaffected. Additionally, greater acetate levels were obtained at the temperature of 28^{o}C compared to a higher temperature of 34^{o}C. The lower temperature of about 25^{o}C also results in a high average molecular mass (Shu et al., 1990).

The molecular conformation of xanthan gum chains in solutions has been well studied and described in the literature. The side-chains are closely aligned with the polymer backbone, which results in right-handed single, double, or triple helix structures. The ordered secondary structure may have one of the conformations - native or renatured. Xanthan's native conformation appears as a five-fold helical molecule, stabilized by inter- and intramolecular hydrogen bonds with a diameter of 1.9 nm and a pitch of 4.7 nm. These structures readily interact with other polymers to form a complex. However, ordered helix structure could be affected by the temperature or salt concentration. At higher temperatures, xanthan gum tends to form random coils. The transition temperature may result in a complete or partial double-strand separation. However, the reverse process, in which the coil is transformed back into the helix is called renaturation and occurs when the temperature drops below the transition temperature. The renaturation is a reversible process, while denaturation of a native form is irreversible (Garcıa-Ochoa et al., 2000; Bejenariu et al., 2008; Petri et al., 2015; Elella et al., 2019). In the aqueous solutions, xanthan gum tends to form a network due to the spontaneous forming of ordered double-helical structures. However, dissolved xanthan gum does not readily form a hydrogel, and the formed weak gel has poor swelling properties (Liu et al., 2015).

3.2. PROPERTIES

The xanthan gum solution's properties strongly depend on its chemical structure, type of salt present in the medium, pH, and temperature (Table 1). However, it could be dissolved in both cold and hot water.

Deacetylated gum created stronger connections with galactomannans (e.g locust bean gum, guar gum) than depyruvated xanthan (Tako, 1991). Additionally, a high degree of acetylation and pyruvilation levels of the xanthan gum resulted in increased water solution viscosity due to the desirable inter-molecular arrangements (Tako et al., 1984). Moreover, depyruvated xanthan gum is more suitable for improved oil recovery (Kano et al., 1993).

It was observed that salt concentration (> 1-2%) slowed down xanthan gum's hydration. As a result, it was necessary to dissolve it before salt addition. Monovalent salts such as sodium chloride affect the viscosity depending on the gum concentration. At higher gum concentrations (> 0.25%), the addition of salt results in increased viscosity. Divalent salts (magnesium, calcium) have a very similar impact on viscosity. On the other hand, trivalent ions promote xanthan gum gelation. Such influence of the salt type on the xanthan properties allows the development of a solution with optimal rheological properties (Petri et al., 2015).

Despite the high stability of the xanthan gum in a wide range of pH (2–11), its viscosity could also be moderated by changing pH. Alkaline medium above pH 9 results in deacetylation. At pH > 4.5, xanthan gum shows a polyanion nature. Acidic environment < 3, on the other hand, leads to depyruvation. However, these viscosity changes are entirely

reversible by neutralization because they result from molecular conformation changes rather than degradation. Reduction of viscosity is an effect of repression of the electrostatic repulsion between gum side chains. Neutralization of carboxylate groups by re-ionization allow the original viscosity recovery. Xanthan gum is soluble in sulfuric acid (5%), acetic acid (5%), nitric acid (5%), phosphoric acid (25%), and it will hydrate up to 5% sodium hydroxide (Rinaudo et al., 2009).

The influence of the temperature on the xanthan gum viscosity is related to the conformational changes and is fully reversible from 10 to 90^{o}C. Xanthan gum viscosity decreases with rising temperature to 40^{o}C, increasing in the range of 40 - 60^{o}C, and declines above 60^{o}C. It is a result of changing the state of the gum molecules from ordered to a disordered state. The addition of any of the salts to the xanthan gum solution can efficiently minimalize the viscosity changes caused by thermal treatment. However, by cooling the solution down, the original viscosity could be recovered. This unique behavior makes the xanthan gum an excellent additive compared to other thickeners (Petri et al., 2015).

4. XANTHAN HYDROGELS

A major advantage of hydrogels is absorbing large quantities of water while remaining insoluble in aqueous solutions. The ability to absorb water arises from the hydrophilic groups attached to the polymeric backbone, while their resistance to dissolution results from the crosslinked network chains. Typically, the mass fraction of water in a hydrogel in the swollen state is much higher than the mass fraction of the polymer, allowing interaction with biological systems. Due to their high flexibility, they can undergo

movement and deformation without significant affection in their form. Other advantages of the hydrogels are reversibility, sterilizability, desired functionality, and biocompatibility (Bejenariu et al., 2008; Ahmed, 2015; Elella et al., 2019).

Developed methods for xanthan gum crosslinking provide stable hydrogels, however, some of them require the use of toxic substances or harsh environmental conditions like high temperature, pressure, or very low pH (Table 2). Ensuring such conditions is, in some cases, necessary during the crosslinking to change the xanthan conformation from double-helical to disordered. By this transition, functional groups of xanthan backbone and side-chains become more accessible for crosslinking agents (Bilanovic et al., 2015). However, in this review, we would like to focus only on methods that can potentially be used in trapping living bacterial cells after applying modifications without significant affection for hydrogel's properties. So far, crosslinked xanthan gum has been used as a carrier for bacterial immobilization only in one study (Dzionek et al., 2021), and it will be discussed later. However, the incorporation of crosslinked xanthan gum into a bioremediation study has promising potential.

Overall, xanthan crosslinking methods that can be used on living cells could be divided based on the bonding location. Xanthan crosslinking with divalent cations, adipic acid dihydrazide, and glycerol takes place on the side-chains monomers. On the other hand, citric acid or trisodium trimetaphosphate results in bonds between monomers from the backbone. Each of the groups has some implications, however, the major issue in most of the methods is a need to change chains conformation to make reactive groups more accessible.

The simplest method to build up the physical network from the xanthan chains is the use of divalent cations. The complex is build up between two pyruvate residues at the end of the side chains (Figure 1A). As a result, intramolecular crosslinking and side chains arrangements occur, and the effect of shear rate on the viscosity level is eliminated. Xanthan gum naturally binds Ca^{2+} ions in order to stabilize the native helical structures. However, to obtain a gel-like state, Ca^{2+} ions should be added at 100% stoichiometric equivalence. The addition of a higher amount of ions, on the other hand, leads to a weaker gel-like state. This phenomenon probably is related to the binding of Ca^{2+} ions to individual carboxyl groups instead of their intermolecular binding sites. As a result, the degree of complexation and network formation decreases (Mohammed et al., 2007; Bergmann et al., 2008). It was observed that the binding of heavy metal ions like Cd^{2+} and Pb^{2+} leads to stronger connections than the lighter cations, such as Ca^{2+} and Mg^{2+} (Bergmann et al., 2008). Such a behavior could be used to separate the metal ions from the wastewater and increase their accumulation in the biopolymer.

In another technique, a xanthan hydrogel could also be synthesized using adipic acid dihydrazide (ADH) as a water-soluble and non-toxic cross-linker. ADH was commonly used for crosslinking polymers like hyaluronan (David et al., 2008), alginate (Bouhadir et al., 2001), or pullulan (Dulong et al., 2006). The use of xanthan gum crosslinking with ADH was performed in the presence of 1-ethyl-3[3-dimethyl amino] propylcarbodiimide hydrochloride (EDCI) to activate carboxylic groups in the xanthan gum. The acid pH favors the protonation of EDCl's nitrogen atoms, which results in creating an unstable intermediate O-acylurea. In the side reaction, it is stabilized into N-acylurea. Later, in the presence of ADH, the amine bonds are created, and the gel is formed

(Figure 1B). Interestingly, the authors tested different experimental conditions to maximize the yield of xanthan gel synthesis. It was noted that the high xanthan concentration (25 g/L), temperature (90^{o}C), and strong stirring lead to the formation of a higher amount of gel. However, the authors suggest that higher temperature only improves the movement of the chains (Bejenariu et al. 2008). Bypassing this issue by prolonging the crosslinking time could probably lead to gel formation at a lower temperature.

The high-temperature requirement for conformation transition was partially resolved by Bilanovic et al. (2016). In this study, xanthan gum was crosslinked by glycerol at room temperature. The partial dissociation of a double-helix to flexible single-stranded coils was achieved by conducting the reaction in a solution with low ionic strength. As a result, carboxyl residues of the xanthan's side chains were exposed to hydroxyl groups in glycerol. The mechanism of crosslinking reactions between glycerol and xanthan gum includes three types of connection. The linkage may be located between carboxyl groups located at adjacent monomers of the same side chain, adjunct side-chain monomers of the separate helix, or side-chain monomers of the separate helix (Figure 1C-D) (Bilanovic et al., 2015). Obtained by this method, the gel was characterized by increased hardness, which could be used in bioremediation systems characterized by high shear forces or packed-bed bioreactors.

An interesting way to crosslink xanthan's backbone monomers involves using polycarboxylic acids like citric acid or malic acid. To obtain a relatively rigid hydrogel with a homogenous and porous structure, there is a need to conduct the process at a relatively high temperature of 90 - 165^{o}C. Interestingly, Li et al. (2019) studied the crosslinking

process at a lower temperature (40°C) but for a more extended period (4 weeks). Moreover, xanthan gum was crosslinked in the solid-state and not dissolved in aqueous media. Obtained results show that after four weeks of incubation with citric acid, the viscosity of the hydrated gel dropped from 11.8 mPa*s to 0.9 mPa*s. Among known mechanisms for xanthan gum crosslinking by polycarboxylic acids, the authors suggest that the bonding process needs a few esterification reactions without a high temperature. A critical step that guarantees proper crosslinking is dehydration of polycarboxylic acid to cyclic acid anhydride, which is more reactive than a carboxylic acid group (Figure 3A). This mechanism occurs only with polycarboxylic acids, which contain at least three carboxylic acid groups. The developed gel was characterized by high acid resistance, which is essential in biodegradation processes in wastewater treatment plants. This feature could extend the carrier's lifetime in these types of harsh environments.

An organic solvent-free xanthan gum crosslinking method based on the xanthan gum backbone alone was proposed by Tao et al. (2016) and Bejenariu et al. (2008) in a strongly alkaline environment. Xanthan network synthesis in alkaline media was well studied. However, it was noted that used cross-linkers were toxic or carcinogenic (Giri et al., 1997). Trisodiumtrimetaphosphate (STMP) is a non-toxic cyclic triphosphate, commonly used to crosslink other polysaccharides like starch (Muhammad et al., 2000). The first step in xanthan crosslinking with STMP strongly depends on the presence of NaOH. In an alkaline environment, STMP is degraded by the opening of the triphosphate cycle. As a result, xanthan grafted sodium tripolyphosphate (STPPg), an intermediate molecule, is formed. STPPg reacts with xanthan alcoholate groups in the next step, and the mixture of mono and diester phosphates is created (Figure 3B). After crosslinking

procedure, xanthan hydrogel needs to be purified with dialysis to remove all remaining STMP molecules and inorganic pyrophosphate. Due to the vulnerability of STMP to pH changes, there is a need to monitor and adjust pH to maintain alkaline conditions during the crosslinking process (Bejenariu et al., 2008; Tao et al., 2016). Obtained by this method, a gel is characterized by a pore size of 114.5 ± 22.1 μm. It was noted that with the increase of xanthan concentration, the pore sizes were decreasing (Tao et al., 2016). Additionally, the swelling properties of XG/STMP hydrogels strongly depended on the STMP:XG ratios, STMP concentration, and phosphate changes (Bejenariu et al., 2008). Adjusting the pH to slightly alkaline conditions should be possible to trap living microbial cells inside crosslinked by this method xanthan gum. Due to the high acid and mechanical resistance of obtained hydrogel has application potential in bioremediation studies.

5. APPLICATIONS OF IMMOBILIZED LIVING CELLS IN BIOREMEDIATION

As one of the immobilization types, encapsulation and entrapment bring many advantages in bioremediation studies. In these methods, microorganisms are enclosed in a polymeric matrix in a way that they can move and grow only within a carrier. Entrapment involves the inclusion of cells in a polymer network or a membrane. In contrast, encapsulated cells are free in the solution but isolated from the environment *via* a thin shell layer. Trapped can be performed in preformed porous matrices, or the porous matrix is created *in situ* around the cells. The primary benefit of these methods is the significant protection of immobilized microorganisms against the external environment, especially other microbial cells. On the other hand, this kind of isolation can lead to nutrients and metabolites exchange limitations. Therefore, mass transfer is one of the crucial factors

influencing the efficiency and activity of immobilized cells. It is worth noting that mechanical stability could be limited and destroys its structure depending on the carrier. Despite this, immobilization *via* entrapment has been shown to have remarkable advantages over non-immobilized microorganisms in bioaugmentation. Cells are protected from fluid shear stress, harsh environment, and cells leaking to the medium is minimal (Dzionek et al., 2016; Partovinia et al., 2018; Ahmad et al., 2020).

There are numerous successful applications of entrapped cells in bioremediation studies at the laboratory scale (Ramteke et al., 2016; Dzionek et al., 2021; Zdarta et al., 2022). Various synthetic (polyvinyl alcohol, polyurethane, polyacrylamide) and natural (chitosan, alginate, carrageenan, agar) polymers have been used for environmental applications. Despite the superior mechanical stability of synthetic polymers, their use in the environment is limited due to their poor biodegradability and biocompatibility. On the other hand, using natural carriers is more suitable, especially in the soil. However, their poor mechanical and chemical stability exclude their use in a harsh environments like wastewater treatment plants (Dzionek et al., 2016; Partovinia et al., 2018).

As mentioned in the previous chapter, xanthan gum is not a popular polymer in bioremediation processes. It was used only as an additive to improve the carrier or immobilized cells properties in most studies. Among commonly practiced immobilization techniques, xanthan gum has been mixed with carriers or microorganisms during the adsorption on the surface or mixed with other polymers during entrapment or encapsulation (Table 3) (Leenen et al., 1996; Song et al., 2005; Kwon et al., 2009; Fernandez et al., 2010; Stelting et al., 2012; McCarthy et al. 2017; Dzionek et al., 2021).

The addition of xanthan gum into carrier polydimethylsiloxane (PDSM) before incubation with *Acidithiobacillus ferrooxidans* (strain DSM11477) resulted in the pore diameter change on the carrier. Pure PDSM was characterized by a smaller amount of larger pores, whereas on the PDSM/XG surface, there were noted the larger amount of smaller pores. This feature was crucial for the viability of the cells in the formed biofilm because cells could not survive in large pores. Biofilm on the PDSM/XG was also thicker than on the pure PDSM, 23 ± 7 μm and 11 ± 3 μm thick, respectively. Authors suggest that the addition of xanthan gum increased the carrier's polarity, which resulted in a higher amount of binding sites on the carrier and, therefore, a better quality biofilm (Fernandez et al., 2010). On the other hand, improving the internal porosity of the carrier could disrupt its integrity and therefore weaken its mechanical resistance. However, this kind of xanthan gum influence on the carrier might increase the bioavailability of cheap non-porous carriers, enabling their bioremediation use.

Interesting studies were conducted by Stelting et al. (2012) and McCarty et al. (2017) during the biofilm formation on the zeolite. Mixing the microorganisms with xanthan gum and coating them onto a zeolite significantly increased the survival rate of the cells. It was observed that strain *Pseudomonas* sp. strain ADP (DSM11735) mixed with xanthan gum remained within 100% survival rate for ten weeks. In contrast, pure ADP suspension was countable during storage in the lysogeny broth medium only for 7-8 weeks. Additionally, during storage in the 25°C and closed container temperature, prepared biocatalyst with xanthan gum was characterized by the survival rate similar to those stored in the 4°C (Stelting et al., 2012). On the other hand, the viability of the strain *Pseudomonas veronii* (strain CIP104663) mixed with xanthan gum dropped from 10^9 to 10^6 cfu/ml but

remained constant for four months in Hg-contaminated soil (55 μg per kg of soil). Simultaneously, *P. veronii* not mixed with xanthan gum showed an immediate and steady decrease in the soil, which resulted in total population collapse after two months (McCarthy et al., 2017). These results showed that xanthan gum could maintain high cell viability for a sufficient time to allow their delivery to contaminated sites. Compared to expensive and difficult to prepare protocols for transporting cells to application sites, immobilization of microorganisms on zeolite and coating them with xanthan gum enables the delivery to such places of a large number of cells in a small volume.

More frequently, xanthan influence on the other carriers and microorganisms has been studied during the entrapment or encapsulation. A fascinating study was conducted by Leenen et al. (1996) regarding the influence of gum's addition into a k-carrageenan or Ca-alginate onto its stability in domestic wastewaters. Pure Ca-alginate and k-carrageenan beads with trapped *Nitrosomonas europaea* (ATCC19718) cells were dissolved within a few days in domestic wastewater due to high ion concentrations. In comparison, the addition of xanthan gum resulted in tripling the lifetime of the gel beads. Dissolution of κ-carrageenan and Ca-alginate in domestic wastewater is a significant issue. However, these relatively cheap and simple bioproducts carry many benefits in bioremediation processes. Thus prolongation of their lifetime in domestic wastewater could improve microorganisms' performance in the most crucial stage of bioaugmentation. The main disadvantage of introducing xanthan gum into wastewater is its poor biodegradability. After the dissolution of κ-carrageenan and Ca-alginate, xanthan gum will be released. Therefore, study into effective ways to degrade xanthan is essential.

Entrapment and encapsulation of bacterial cells, despite the achievement of bead characterized by high mechanical strength, also represent some disadvantages such as cell leakage and cell loading limitation. However, some of these features could be improved by adding natural gums. Xanthan gum has been mixed with poly(vinyl alcohol) (PVA) in the entrapment of *Ochrobactrum anthropi* SY509 in order to study biological denitrification. Moreover, Tween 20 (0.2%) was added to polymers to improve the permeability of the membrane wall. The addition of the surfactant resulted in decreasing the number of floating beads on the medium surface. Furthermore, it was noted that xanthan gum enhanced beads' surface properties and lowered aggregation rate. In this study, the authors proved that the addition of xanthan gum minimized cell leakage. They found approximately 1.8% of initially immobilized cells per bead after 24 h incubation in the packed bed bioreactor. However, the released cells were hardly detected in the effluent wastewater. During the denitrification test with hybrid-immobilized beads, it was observed that nitrate-N (100 mg/L) was degraded in 45 min, which was the fastest degradation in this study. On the other hand, PVA beads with calcium alginate needed 120 min to remove the same amount of nitrate-N. In contrast, immobilized beads containing only PVA degraded only half of the nitrate-N during 180 min (Song et al., 2005). Due to the significant problem with obtaining a proper pore size in entrapped or encapsulated products, appropriate filler could improve many bioproducts. Minimizing cell leakage from the entrapped beads may also benefit GMO immobilization, which in many countries cannot be released into an environment. Xanthan gum is known for its holding capacity. This feature also allows more bacterial cells to be packed into beads. As a result, faster biodegradation can be achieved.

Despite the surfactants, complex PVA/XG has also been merged with activated carbon. Among all tested trapping polymers (PVA + xanthan gum (XG) or alginic acid; k-carrageenan; sodium alginate; chitosan), it was noted that the excellent spherical bead shape and strength characterized PVA/XG beads with trapped *Pseudomonas fluorescent* and activated carbon. However, a small amount of cell leakage and bead agglomeration was observed during bead formation. In the case of PVA/XG beads, phenol degradation was complete after 30 h (100 mg/L), and it was the fastest biodegradation among all tested variabilities (Kwon et al., 2009). It was suggested that PVA/XG with activated carbon and microorganisms developed a cooperative phenol removal system with biological and physical treatment. Trapped cells of *P. fluorescent* were degrading phenol, whereas activated carbon was adsorbing it and simultaneously decreasing its negative impact on microorganisms.

So far, only one study presents a method and properties of crosslinked xanthan gum as a basic trapping polymer (Dzionek et al., 2021). The patented carrier was preformed by crosslinking xanthan gum with adipic acid dihydrazide (ADH) in the presence of 1-ethyl-3[3-dimethyl amino] propyl carbodiimide hydrochloride (EDCl), by the method proposed by Bejenariu et al. (2008), but with reducing toxicity for microorganisms modifications. To improve the mechanical properties of the biocatalyst, after trapping *Bacillus thuringiensis* B1(2015b) or *Planococcus* sp. S5, it was coated with polydopamine (PDA). After the immobilization procedure, the bacterial survival rate analysis revealed that it was neutral for strain B1(2015b). In contrast, for S5 cells, this method was too harsh, and the survival rate was decreasing after prolongation of the coating time and increase of xanthan concentration. Developed hybrid XAN/PDA was characterized by Young's modulus 0.26 ±

0.04 or 0.42 ± 0.06 MPa for B1(2015b) or S5 strains, respectively, and high stability in the acidic and neutral environments. Trapped cells of B1(2015b) remained 99% alive after 35 days of storage in 0.9% NaCl solution in 4°C. Additionally, a short-term naproxen biodegradation study was performed with autochthonous microflora from a wastewater treatment plant and bioaugmented XAN/PDA/B1(2015b) composites. Observed naproxen (1 mg/L) biodegradation was the fastest among the documented in the literature. This phenomenon resulted in an enhanced reduction of chemical oxygen demand (COD) from the wastewater by autochthonous microflora (Dzionek et al., 2021). This study shows that after applying modifications to existing methods for xanthan gum crosslinking, this gum has the potential for immobilization of living cells. Due to its non-biodegradability and high chemical and mechanical resistance, developed bioproducts have enormous potential for bioaugmentation of one of the harshest bioremediation systems in wastewater treatments plants.

6. XANTHAN GUM REMOVAL

Xanthan gum overall is considered non-biodegradable due to its non-typical conformation and monomers. Introducing such material into an environment has its pros and cons. High mechanical and chemical stability is an essential feature for a carrier to provide a safe micro-environment for immobilized microorganisms. In contrast, after complete bioremediation of an ecosystem, a carrier should not accumulate in the environment. Despite the xanthan gum outstanding properties and potential in bioaugmentation, we would like to focus on a documented ways of its degradation, biodegradation or transformation methods.

Many xanthan gum degradation and biodegradation techniques have been developed. Physical methods include ultrasounds, radiation, chemical treatment, and high temperature. Biological methods are based on crude extracts from microorganisms or living cells systems. Due to the dense and packed structure of xanthan gum in a temperature below 70-80^{o}C and oins-rich medium, its degradation is limited. However, changing its conformation from helical into unordered could potentially improve the degradation efficiency.

6.1. PHYSICAL DEGRADATION

The ultrasonic method is the most ecofriendly, rapid, effective, and generate less toxic byproducts among all xanthan physical degradation method. Polymer exposure to high-energy ultrasonic irradiation results in a permanent reduction in its molecular weight. Interestingly, there are two hypotheses on how the ultrasonic chain scission mechanism work. First assumes that cleavage does not occur in a random location on the polymer but at the center of the polymer chain. Second, the midpoint model assumes that polymer degradation is followed by a random cleavage, where chain breakage occurs randomly until the limiting molecular weight is reached. The smallest oligomers from xanthan gum obtained by ultrasonic treatment in an aqueous environment were achieved at sonication intensity 11.5 W/cm^2, lasting 120 min in a salt-free solution. Oligomers were characterized by molecular weight around 3.2 x 10^5 g/mol. The authors noted that NaCl significantly affects the xanthan degradation rate. When the concentration of NaCl was increasing, the degradation rate was decreasing. This phenomenon is related to the fact that in high ions concentration, xanthan gum changing its conformation to dense and packed helixes, which

are difficult to access by irritating agents. Additionally, it was proved that a random scission model followed xanthan degradation (Saleh et al., 2017).

Xanthan degradation by exposure to γ-rays can be performed in two ways, in a solid or aqueous state. Degradation in the solid state was performed by Şen et al. (2016), and it was noted that it results only in the scission on main-chains of the polymer. Side-chains remained unaffected. However, the degradation was more effective when the dose of radiation was decreasing. The lowest molecular weight of the products was equal to 1 x 10^6 g/mol and 20 kDa and was obtained at a dose of 0.1 kGy. Probably, this was an effect of enhanced oxidative degradations during irritation. Interestingly, despite the molecular weight changes, radiation-induced degradation did not change the non-Newtonian and shear-thinning behavior of xanthan gum. As a continuation of this study, Hayrabolulu et al. (2018) presented the γ-rays induced xanthan degradation in an aqueous state. They found out that dissolved xanthan was more susceptible to radiation than in solid-state. At a radiation rate of 3.3 kGy/h, oligomers with a molecular weight of about five kDa were observed. As an effect of γ-rays, hydroxyl radical and hydrogen atoms are formed. Subsequently, they can abstract hydrogen atoms from the polysaccharides and form macroradicals. These macroradicals can perform chain scission, inter- and intramolecular recombination, hydrogen transfer, and disproportionation of macroradicals.

An interesting study was conducted by Nnyigide et al. (2021) about potential xanthan degradation abilities by most common denaturants in biotechnology - urea, sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB), due to their abilities to disrupt the native structure of macromolecules. The authors observed that CTAB has the most significant degradation effect on the xanthan gum among all tested substances. This

could be explained by the expulsion of water molecules from the hydrogel and the incorporation of CTAB in the xanthan gum side chain. As a result, the hydrogen bonding and other noncovalent interactions were disrupted, and it was observed a viscosity drop. In contrast, urea and SDS did not interact with xanthan side chains.

The influence of high temperature on biodegradable and edible films, which consist of maize or wheat starches and xanthan gum, was studied by Soares et al. (2005). Authors tested a different starches/xanthan ratios in films (100:0, 70:30, 50:50, 30/70, 0/100 (w/w)) and degradation temperatures in the range from 25 - 500^{o}C. The biggest weight loss was observed at temperatures between 275 and 360^{o}C, both for pure xanthan and starch blends and also for mixed biofilms. The study revealed that xanthan gum was less thermally stable than starch from different sources. Higher residual weight after xanthan degradation (39 – 42%) could be a result of the presence of Na^{+} in the structure, which could form inorganic intermediates during degradation. The thermal degradation mechanisms of xanthan begin with cleavage of bonds in the side chains with subsequent scission of the main chain.

The most promising method for xanthan gum removal from the industrial scale bioremediations systems like in wastewater treatment plants seems to be degradation by ultrasounds. Mohammadi et al. (2011) presented a method for excess sludge reduction in biological wastewater treatment based on the ultrasound. The sludge was separated from the wastewater, treated with ultrasonic waves, and returned to the reactor. Comparing the generated frequency of ultrasound waves for excess sludge reduction with that generated for efficient xanthan gum degradation (Saleh et al., 2017), we could assume that 20 kHz will be sufficient for both processes. Simultaneously separation and treatment of excess

sludge and xanthan beads could significantly lower the cost of the xanthan beads degradation process without affecting the effectiveness of excess sludge removal. Returning wastewater into the bioremediation system after the ultrasound treatment could also be beneficial. Naddeo et al. (2009) proved that sonication increased the biodegradability of pharmaceuticals (diclofenac, amoxicillin, carbamazepine) in wastewaters and helped obtain a zero discharge at the final effluent. Despite that, the authors suggest testing the toxicity of treated wastewaters due to the possibility of creating more toxic degradation products.

6.2. BIOLOGICAL DEGRADATION

In the natural environment, xanthan degrading microorganisms are rare due to the not typical covalent bonds between sugar monomers. However, there are described in the literature a few bacteria species that were able to degrade xanthan gum. All of them were isolated from the soil or sewage sludge.

The first report describing a xanthan-degrading microorganism was published by Cadmus et al. (1982). The strain identified as *Bacillus* sp. 13-4 was isolated from the sewage sludge. It was observed that strain produced a xanthanase with an activity of 137.84 U. This enzyme was stable up to 42^{o}C and over the pH range of 4.8 to 6.0 and was proved to catalyze the scission reactions only on the xanthan gum's side chains. Biodegradation of xanthan (0.15%) by crude extract with xanthanase after 24 h resulted in 35 - 40% loss of its initial concentration. In the medium were found D-glucuronic acid, D-mannose, pyruvylated mannose, 6-O-acetyl D-mannose, and glucan as a degradation products.

Additionally, the authors found that microorganisms can affect xanthan stability when the contact period in open containers ranges from one month to one year.

Xanthan lyase, a second enzyme that degrades only xanthan side chains, was isolated from soil bacteria *Paenibacillus alginolyticus* XL-1. This enzyme was characterized by the stability in the 30 - 35°C and acidic pH and vulnerability to the presence of specific salts (0.1 mM $CuCl_2$ caused a 70% drop in the activity; 20.1 mM HgCl – 100% drop; 85 mM NaCl - 40% drop). Interestingly, strain *P. alginolyticus* XL-1 was able to use xanthan as sole carbon source (Ruijssenaars et al., 1999).

Hou et al. (1986) isolated a consortium that was able to degrade xanthan in the soil. Most of the attention was paid to the strain *Bacillus* sp., which was able to degrade xanthan in the presence of 4% NaCl. However, this strain was not able to grow without a second strain (not identified in the study), which stimulated xanthan degradation. Mixed culture was able to reduce the viscosity of the medium mixed with xanthan (2.5 g/L) from 400 mPa·s to 30 mPa·s within 24 h. In the medium, there were present degradation products: glucuronic acid, pyruvated mannose, glucose, mannose, and acetylated mannose. The degradation mechanism by strain *Bacillus* sp. was different from observed by Cadmus et al. (1982) because enzymes from this strain could cleavage side-chains and the main chain of the xanthan.

Another isolated strain from the soil was *Cellulomonas* sp. LX. It was observed that this strain was responsible for xanthan (0.05%) viscosity drop to almost 0 after 30 h of incubation. The authors found out that xanthan degradation was inhibited when the glucose was added. They suspected that side- and main-chains of the xanthan were degraded due to

the presence of oligosaccharides. Interestingly, the optimum temperature for xanthan degradation by crude enzyme extract was 40°C and pH 6.0 (Liu et al., 2005).

The first xanthan biodegradation in the activated sludge was performed by Muchová et al. (2009). In the beginning, there was a 100-hour long acclimation phase, where no xanthan degradation was observed. This study showed that the number of bacteria that could degrade xanthan was not too high in that phase (4.5 x 10^3 cells/ml). Interestingly, only one isolate was able to degrade xanthan as a sole carbon source. It was a slow-growing psychrophilic bacteria, gram-variable and spore-forming aerobic rod, which produce extracellular depolymerase, which degrades xanthan. This strain was identified as *Paenibacillus* sp. XD. Enriched with this strain consortia were able to complete the removal of xanthan gum (0.5 g/L) in almost 100 h of incubation.

In the literature, there is present only one degradation study regarding a crosslinked xanthan gum. A degradability of the hydrogel based on the xanthan gum graft copolymerized with polyacrylic acid was checked by the soil burial method. Samples were buried at a depth of 5 cm and harvested every 7th day for 70 days. A gradual weight loss was observed during the incubation, with a maximum after 70 days and equal to 78.3% (1.11% per day). SEM micrographs clearly showed that the surface of obtained composites was highly grooved with a veins-like network developed by soil microbiota (Sukriti et al., 2017).

7. FUTURE PERSPECTIVES

It has been known that dynamic and harsh environmental conditions characterize bioremediation systems. The presence of various metabolites, xenobiotics, and ions affects

the integrity of the carriers and the viability of bioaugmented strains. Therefore, the chemical and mechanical stability of the carrier is the most crucial factor in the design of bioremediation studies. On the other hand, effective xenobiotic biodegradation results from viable cell actions, for which crosslinking procedures were not significantly affected during entrapment. Achieving both of these goals is challenging.

Modifying already developed methods for polymers crosslinking could be a solution for this issue. Understanding the structure of the polymer and possible ways to crosslink its chains allows developing protocols safe for vulnerable microbial cells. Due to the popularity of xanthan gum in biomedicine and pharmacology, methods for its crosslinking are well described in the literature. Unfortunately, xanthan gum influence on the living microbial cells is not known. Understanding these interactions could help prepare better bioproducts for harsh bioremediation systems, like these in wastewater treatment plants.

Methods described in this review have the most promising potential to be modified to minimize the influence on the microbial cells and simultaneously result in a carrier characterized by high mechanical and chemical stability. However, those are theoretical projects that need to be investigated and tested in the laboratory scale bioremediation systems. The first attempt to modify the xanthan gum crosslinking procedure and trapping *Bacillus thuringiensis* B1(2015b) and *Planococcus* sp. S5 was successful and showed its potential use in wastewater treatment plants. However, the more specific characterization of formed bonds and their influence on the bacterial cells is essential.

Among known methods for xanthan gum degradation, none of them were tested on the crosslinked forms of the gum. Some of them, like ultrasounds, could be potentially

applied in the industrial scale bioremediation systems. By modifying already formed systems, the costs of the xanthan beads degradation could be minimized. The influence of degradation processes on the crosslinked xanthan gum deserves to be investigated.

8. CONCLUSIONS

Although the broad uses of conventional polymers for living cells entrapment, there is a need to search for their natural equivalents with better properties. Xanthan gum is considered one of the most promising biopolymers in bioremediation studies due to its advantages, like bacterial origin, good mechanical and chemical stability, and stability in the wastewater environment. Presented in this review, methods for xanthan gum crosslinking and their potential modifications demonstrated that the development of entrapment procedures that are safe for live cells is possible. Additionally, despite hard-biodegradable structure, there are ways to eliminate xanthan gum from the environment after successful bioremediation.

9. FUNDING SOURCES

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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10. Figure Captions

Figure 1. Xanthan gum structure (Dzionek et al., 2021; modified).

Figure. 2. Cross-linked side-chains of the xanthan gum by divalent cations (A); adipic acid dihydrazide (B); glycerol (C, D) (Bergmann et al., 2008; Bejenariu et al., 2008; Bilanovic et al., 2015; modified). Xanthan structure was simplified to increase scheme readability.

Figure 3. Crosslinked backbone of the xanthan gum by the rest of citric acid (A) and phosphate residue from trisodium trimetaphosphateadipic (B) (Tao et al., 2016; Li et al., 2019; modified). Xanthan structure was simplified to increase scheme readability.

11. Tables and Figures

Table 1. Properties of commercial xanthan gum. Viscosity measured at C_P=1 g*L^{-1}, T=25°C (Garcıa-Ochoa et al., 2000; modified).

Content	Min	Max
Viscosity (cP)	13	35
Moisture (%)	8	15
Ash (%)	7	12
Nitrogen (%)	0.3	1
Acetate residues (%)	1.9	6
Pyruvate residues (%)	1	5.7
Monovalent salts (g*L^{-1})	3.6	14.3
Divalent salts (g*L^{-1})	0.085	0.17

Table 2. Frequently used methods for the production of the xanthan hydrogels.

Crosslinking agent	Possible application to living cells	Environmental conditions	Properties	Original application	Reference
Divalent cations	Yes	Temperature: 80°C Time: no data pH: no data Salt: 10 mN or 0.3 M NaCl or Me_4N^+; 0–100 mN $CaCl_2$ Additives: -	Weak-gel network	Sorption of salts and heavy metals	Mohammed et al., 2007; Bergmann et al., 2008;
1-butyl-3-methylimidazolium chloride (toxic)	No	Temperature: 120°C Time: 12 h pH: no data Salt: - Additives: -	Good mechanical resistance Good elasticity Thermally induced shape-memory effect	Sorption of salts and heavy metals	Izawa et al., 2010
Acrylic acid & 2-hydroxyethyl methacrylate (toxic)	No	Temperature: 80°C Time: 4 h pH: no data Salt: -	Superporous Good thermal stability High water absorption capability	Controlled drug delivery systems Tissue engineering	Gils et al., 2009

		Additives: APS; N,N,N',N'-tetramethylethylenediamine	Biodegradability		
Adipic acidic dihydrazide	Yes	Temperature: 90°C Time: 24 h pH: 3 Salt: 10^{-3} M $LiNO_3$ Additives: 1-ethyl-3[3-dimethyl amino] propylcarbodiimide hydrochloride	High gel density High water absorption capability Heterogeneous gel structure High pH and thermal resistance	Controlled drug delivery systems	Bejenariu et al., 2008
Citric acid	Yes	Temperature: 165°C/40°C Time: 7 min / 4 weeks pH: no data Salt: - Additives: -	High water absorption capability High acid resistance High porosity	Controlled drug delivery systems	Bueno et al., 2013; Li et al., 2019
Epichlorohydrin (toxic)	No	Temperature: 60°C Time: 3 h pH: alkaline Salt: - Additives: 80% ethanol	Strong viscoelasticity Good thermal stability Good thixotropy	Enhancement of oil and gas recovery	Zhang et al., 2017

Glycerol	Yes	Temperature: 22.5°C Time: 10 min pH: no data Salt: - Additives: -	High water absorption capability Good sorption properties Increased hardness	Food and pharmaceutical industries Slow-release matrices	Bilanovic et al., 2016
Polyvinyl imidazole (toxic)	No	Temperature: 50°C Time: 2 h pH: no data Salt: - Additives: potassium persulfate; N,N'-methylenebisacrylamide	Efficient regeneration ability Antibacterial activity High surface porosity	Dyes sorption (crystal violet) Inhibition of *E. coli* growth	Elella et al. 2019
Sodium trimetaphosphate	Yes	Temperature: 90°C/37°C Time: 24 h / 3 h pH: 13 Salt: 10^{-3} M $LiNO_3$ / - Additives: -	High water absorption capability High acid and mechanical resistance Good sorption properties High surface porosity	Controlled drug, nutrition ingredients, therapeutic agents or cells delivery systems Tissue engineering	Bejenariu et al., 2008; Tao et al., 2016

Table 3. Summary of bioremediation studies that involved xanthan gum addition or use as a base polymer.

Carrier	Immobilized microorganism	Xanthan gum mixed with	Advantages	Disadvantages	Potential application	Reference
Polydimethylsiloxane (PDSM)	*Acidithiobacillus ferrooxidans* (strain DSM11477)	Carrier	Changed pore diameter (higher amount of smaller pores) The increased polarity of the carrier Increased internal and external porosity	Higher porosity weakened the carrier integrity	Increasing bioavailability of cheap polymers with low-porosity, thus facilitating the biofilm formation	Fernandez et al., 2010
Zeolite	*Pseudomonas* sp. strain ADP (DSM11735) *Pseudomonas veronii* (strain CIP104663)	Microorganisms suspension	Increased survival rate during storage at 25°C Cheap microorganism delivery system	-	Transport of high amount of microorganism in a small volume Long-term storage in a room temperature of ready-to-go	Stelting et al., 2012; McCarthy et al., 2017

					bioproducts	
κ-carrageenan/Ca-alginate	*Nitrosomonas europaea* (ATCC19718)	Carrier	Improved chemical stability in wastewater	Introduction of hard-biodegradable polymer into wastewater	The decreased dissolution rate of k-carrageenan/Ca-alginate beads in domestic wastewater	Leenen et al.(1996)
Poly(vinyl alcohol) (PVA)	*Ochrobactrum anthropi* SY509	Carrier	Lowered aggregation rate Enhanced surface properties Minimized cell leakage	Reduced mechanical strength of beads	Improving entrapped bioproducts characterized by high cell leakage properties Entrapment of GMOs for environmental purposes	Song et al., 2005
Poly(vinyl alcohol) (PVA) with activated carbon	*Pseudomonas fluorescent*	Carrier	The more spherical shape of beads Minimized cell leakage Improved	Reduced mechanical strength of beads Limited mass transfer	Bioremediation of highly contaminated sites	Kwon et al., 2009).

			porosity Higher concentration of microorganism in beads			
Xanthan gum with polydopamine	• *Bacillus thuringiensis* B1(2015b) • *Planococcus* sp. S5	-	Reduced toxicity for microorganisms during crosslinking Improved mechanical and chemical stability Elongated survival rate during storage	Not suitable for small bacterial cells Additional hardening required	Bioaugmentation of bioremediation systems in wastewater treatment plants	Dzionek et al., 2021

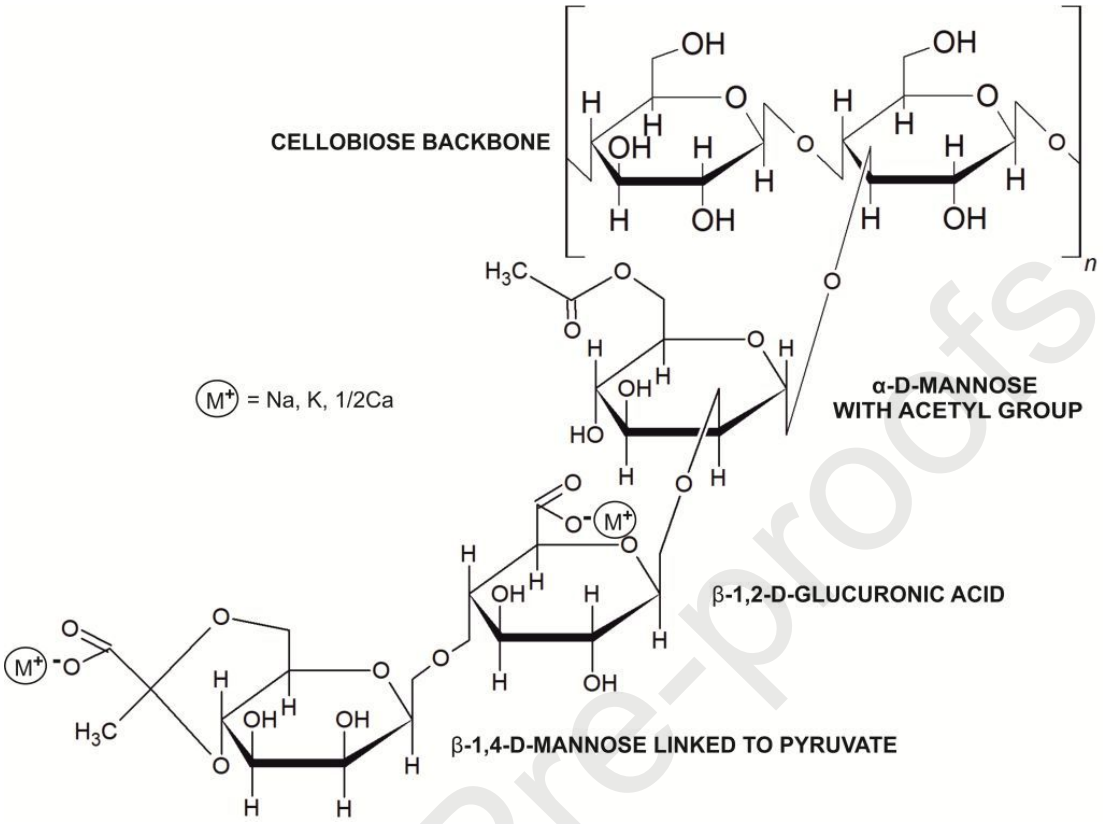
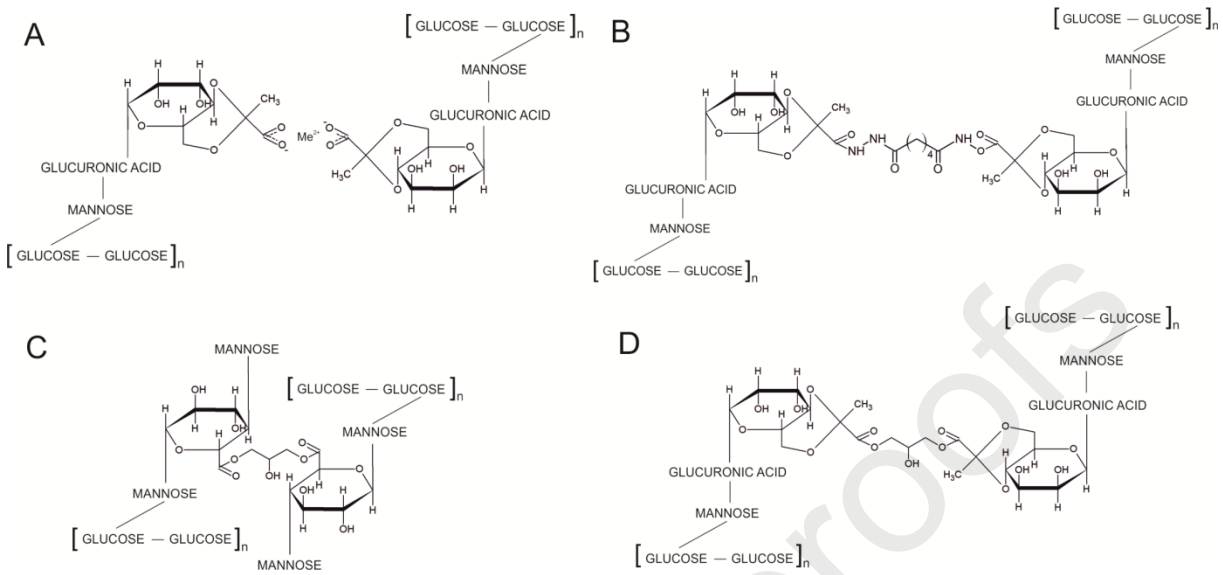


Figure 1. Xanthan gum structure (Dzionek et al., 2021; modified).



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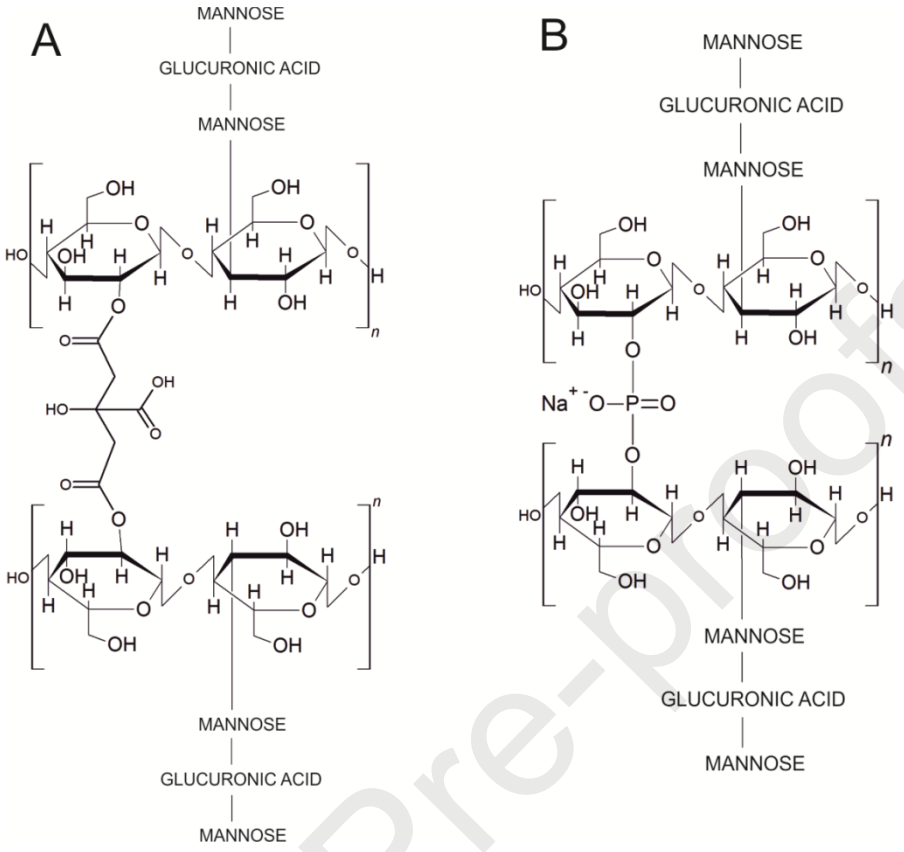

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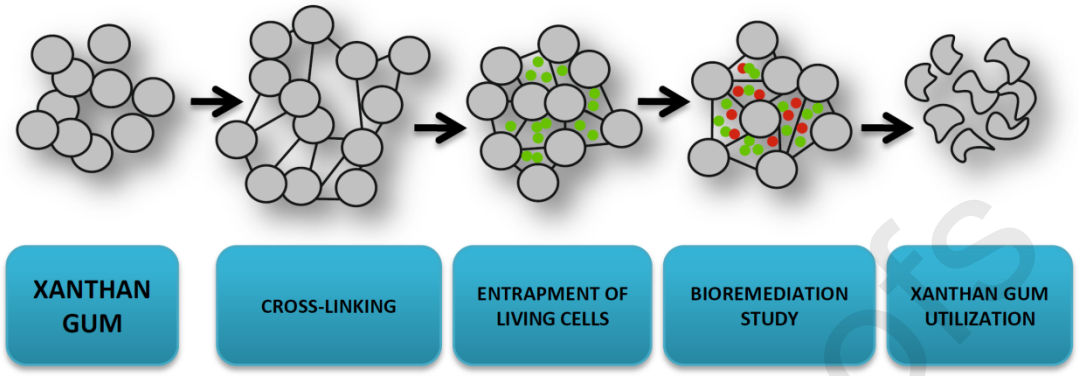

- Xanthan gum unique properties could be used in bioremediation
- Methods for xanthan cross-linking can be adjusted for biological entrapment
- Previous use of xanthan as an enhancer of immobilized cells revealed its potential
- Despite the not typical structure of xanthan chains, there are ways to utilize it