Microorganism Based Biopolymer Materials for Packaging Applications: A Review

Urška Vrabič Brodnjak*

University of Ljubljana, Faculty of Natural Sciences and Engineering, Department of Textiles, Graphic Arts and Design, Askerceva 12, S1-1000 Ljubljana, Slovenia

Abstract: Polymers which are produced naturally or genetically from microorganisms, have a great potential in many fields of material science. One of them are coatings and films which can also be used in packaging materials (papers, boards, cardboards). Polymers like bacterial cellulose, Xanthan, Curdlan and Pullulan are polymers, produced from different bacteria, with specific properties. This group of polymers consists mainly from polyhydroxyalkanoates. Hyaluronic Acid, Poly (gamma-glutamic acid), Cyanophycin and Microbial Exopolysaccharides are well known polymers, but not commonly used in packaging field. Their applications are promising but still under research and implementation. One of the main drivers and the goal for the development of bio based coatings from different bio based polymers, is the production of materials which are fully biodegradable, made from the renewable raw materials. Fermentation of agricultural products produced by bacterial microorganisms causes the production of polymers as substrates. The applications of this products could lead to new possibilities for improving base materials, substrates such as papers, boards and cardboards. The applicability into the coating industry is still related with performance, processing and costs of such polymers. The review paper introduces new possibilities of polymers made from microorganisms, which have a potential in a coating industry.

Keywords: Bio based coatings, packaging, polymers, microorganisms.

1. INTRODUCTION

Packaging research is to develop the use of bio-plastics which are useful in reducing waste disposal and are good replaces of petroleum and a non-renewable resource with diminishing quantities. Among used packaging materials such as paper, plastics, metal (aluminium) and glass, paper is the most used and popular material [1-5]. Paper, board and cardboard are widely used packaging materials in many shapes and products (boxes, folding cartons, corrugated boxes, paper bags, cups etc.) and their biodegradability, barrier and mechanical properties are of prime importance. Packaging paper materials consist of a porous cellulose structure, made of microfibrils, composed of a long chain cellulose molecules in a crystalline and amorphous regions [6]. Cellulose has a hydrophilic nature, because of the presence of the OH groups in the basic unit of cellulose. It is known that the cellulose fibres are porous and therefore the paper easily absorbs water and moisture from the environment and also from the food. Moisture migration can occur in the paper by diffusion of water vapour through the void spaces and also through the fibre cell walls [7]. For the packaging, paper is excellent material, because of its biodegradable nature, lightweight and has sufficient mechanical properties [8]. To achieve good barrier properties, which are important for packaging materials, the functionalities of the paper should be changed. The wetting and barrier properties (i.e., the gas, oxygen, microbial properties) are highly relevant to retain the barrier and mechanical properties of the package and the packed good under certain environment conditions.

Natural polymers are already used as barrier coatings on paper packaging materials, which reduce importation of petroleum and it's derivate into the paper coating industry. In recent years there has been a research focus on renewable biopolymers used as edible films and coatings.

Polymers which are produced naturally or genetically from microorganisms, have a great potential in many fields of material science. One of them are coatings and films which can also be used in packaging materials. Polymers like bacterial cellulose, Xanthan, Curdlan and Pullan are polymers, produced from different bacteria, with specific properties. This group of polymers consists mainly from polyhydroxyalkanoates [1]. Lately Hyaluronic Acid, poly-(gamma-glutamic acid), Cyanophycin, Microbial Exopolysaccharides with which considerable applications in the field of packaging could be made. Their development and applications on different substrates are under extended research. One of the main drivers and the goal for the development of bio based coatings from different bio based polymers, is the production of materials which

*Address correspondence to this author at the University of Ljubljana, Faculty of Natural Sciences and Engineering, Department of Textiles, Graphic Arts and Design, Askerceva 12, S1-1000 Ljubljana, Slovenia; Tel: + 386-1-200-32-82; Fax: + 386-1-200-32-00; E-mail: urska.vrabic@ntf.uni-lj.si

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are fully biodegradable, made from the renewable raw materials. Fermentation of agricultural products produced by bacterial microorganisms causes the production of polymers as substrates.

The applications of this products could lead to new possibilities for improving base materials, substrates such as papers, boards and cardboards. Mechanical, barrier and many physicochemical properties of the packaging materials could be improved. The applicability into the coating industry is still related with performance, processing and costs of such polymers. Environmental indicators are the key issues for responsible production of packaging materials. The review paper introduces possibilities of polymers made from microorganisms, which have a potential in a paper coating industry. The aim of this study was to review the possibilities of such polymers, investigated as potential paper coatings, to summarize the functionalities of certain coatings, to discuss about potential applications, and future prospects for bio-based coatings, produced from microorganisms and use on packaging materials.

2. BIO-BASED COATING MATERIALS

Bio-based polymers are polymers which can be directly extracted from biomass (polysaccharides, proteins and lipids), polymers which are synthesized from bio-derived monomers (polylactic acid – PLA or other polyesters) or polymers which are produced directly by microorganisms (polyhydroxyalkanoates – PHA, bacterial cellulose, xanthan, pullulan and curdlan) (Figure **1**). They can be used alone or in combination. The choice of certain coatings depends on desired function of used material. Bio based polymers are usually coated on papers and cardboards, applied with different coating techniques (solutions coating, surface sizing, curtain coating or compression molding).

At polysaccharides mostly used are chitosan, starches (rice, wheat, maize, corn and potato), cellulose and alginates. They are nontoxic, widely used and available in the market also as waste materials. With great gas, aroma and grease barriers, such coatings have great potential in packaging materials. Because of their hydrophilic nature, polysaccharides exhibit poor water vapour barrier [9, 10].

Proteins are present in plants and animals as the structural and functional material, which is important for their biological activity. They have excellent film forming properties or coatings on paper in various applications for vegetables, meat and fruit packaging's. Usually such coatings are applied by spray or bar coating, even extrusion. Because of its intrinsic

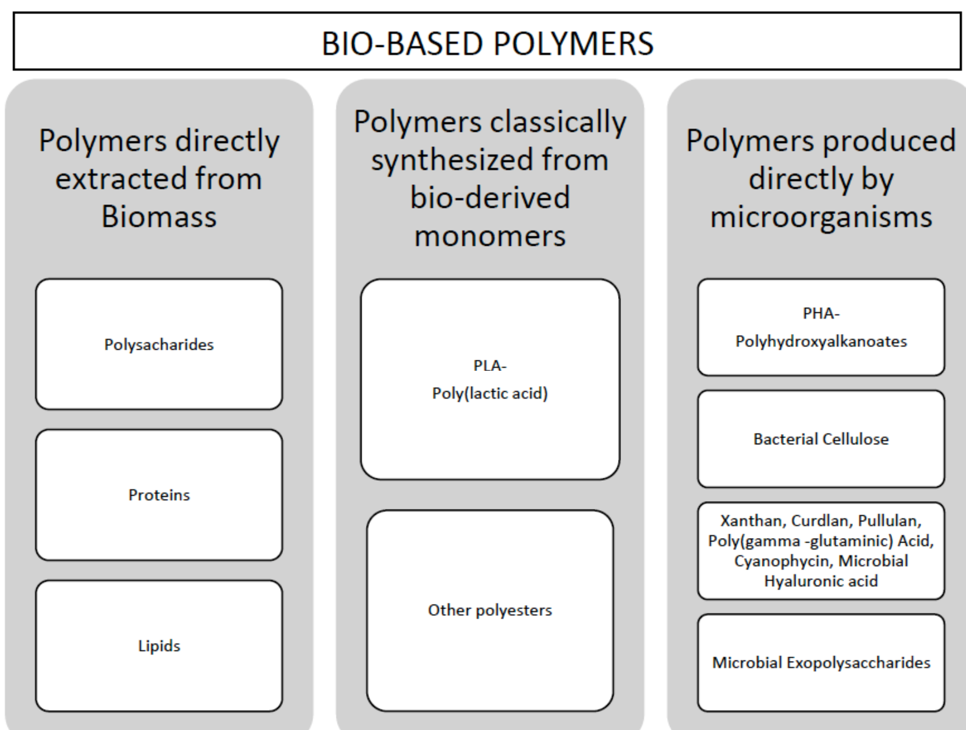

Figure 1: Bio-based polymers used in packaging [8].

hydrophilic property, protein coatings have low oxygen permeability and high water vapour permeability [11].

Lipids such as fatty acids or waxes, could be used as coatings [12]. Waxes have high hydrophobicity and are mostly used in food and drink packaging, to increase water resistance and shelf life of certain product. Lipid coatings have great moisture barrier, but on the other hand they exhibit brittleness, presence of cracks and pinholes on the coatings [13]. Common are composite coatings or multilayer coatings with other bio-based polymers like corn zein, chitosan etc. [6].

Polymers, which are classically synthesized from bio-derived monomers still have high costs, but are lately used in paper and packaging industry. Among them the most used is PLA, because of its accessible production, obtained from the fermentation of renewable materials like sugar, sulphite liquors, agricultural waste products etc. [14, 15]. PLA is thermoplastic polymer, which can be produced on extrusion equipment. It is lactic acid, produced by the anaerobic fermentation, resulting in the mixture of L- and D-lactide acid monomers. Crystallinity and therefore mechanical properties of PLA depend on the presence of L-and D-lactide [16]. L-PLA exhibits higher crystallinity, compared to D-PLA. From the literature it is known that PLA has low oxygen and carbon dioxide barrier, but sufficient moisture barrier. With PLA paperboards were coated with bar coating technique, where water vapour permeability and water absorptiveness decreased, but water contact angle increased [16].

At packaging materials, barrier properties (gas, oil and water vapour permeability) are crucial, because the critical compounds can migrate thorough the material and destroy or degrade food quality. Several biodegradable polymers are widely used in many fields of materials science (also paper coatings), while others are in various stages of development.

Paper coatings which are used in packaging should fulfil requirements such as:

- High barrier properties,
- Great mechanical properties,
- Microbial stability,
- Simple production technology,
- Low cost materials.

In the following paragraphs biopolymers, which are produced directly from microorganisms, used as the paper coatings will be specifically discussed.

3. POLYMERS PRODUCED FROM MICROORGANISMS

In paper, packaging and coating industry plays an important role the development of practical processes and products made from green, biodegradable polymers, which can be used on different substrates. For the most packaging, low water vapour and oxygen permeability are required, to require good food quality.

Polymers, which are produced from microorganisms, have a unique and few also a superior physical properties.

3.1. PHAs or Polyhydroxyalkanoates

PHAs or polyhydroxyalkanoates (Figure **2**) are bio-based polyesters, produced by several micro-organisms and can be accumulated to high levels in bacteria [17, 18]. One of the bacteria is *Pseudomonas putida* [18]. In recent years it has been investigated by many material, chemical, biotechnological etc. scientist, in order to make an application of PHA as bioplastics, printing and photographic materials, biomaterials, applications in medicine like drug delivery carriers and even in biofuels [19]. It is produced by a large scale microbial fermentation, where the production involves strain development, flask optimisations, pilot fermentor studies and at the end industrial scale up [20]. Because of diverse structure and properties PHA is very useful as polymeric materials as bioplastics, fibres, film, biomedical implants etc. [21-23]. In packaging industry it can be used as a material for packaging which is used for short period of time, like packaging for food, shopping bags, paper coatings, cups, upholstery, shampoo bottles, and compostable bags. It can also be used as film, in electronic appliances. PHA materials have a great properties regarding colouring, because it easily stained, therefore it can be used in the printing and photographic industry [24, 25]. Coatings with PHA on paper could be combined with other natural polymers like polysaccharides or cellulose. To provide cost efficient paper coating, blending PHA with mentioned polymers, could make a great solution, to reduce the costs without the loss of sustainability. Since last years, PHA has been investigated in large scale, the future prospects of low cost PHA will become available in coming years. The production should be oriented in the interdisciplinary knowledge from agriculture, packaging industry, biofuels, chemicals, medicine etc.

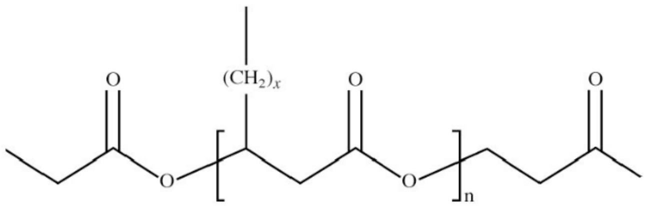

Figure 2: Chemical structure of PHA [19].

3.2. Bacterial Cellulose

Bacterial cellulose (Figure **3**) is currently in the phase of research, progress and implementation into coatings. It is produced by a microbe named *Acetobacter xylinum* [2]. From the previous research it was confirmed that this kind of bacteria is found in rotten fruits and vegetables [26]. There are reasons and predictions why microorganisms generate cellulose. One of them are that the aerobic bacteria produces pellicle to maintain the position to the surface of culture solution and another is that bacteria generates the cellulose in order to protect "themselves" from ultraviolet radiation [27, 28].

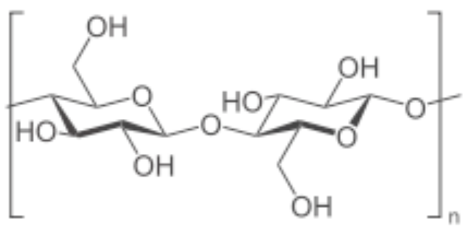

Figure 3: Chemical structure of cellulose [27].

The main advantage of this polymer is a high degree of polymerisation, high crystallinity index and better water holding capacity. Recently bacterial cellulose is used in other fields of material science like textiles (wound dressings, clothing materials for burn wound patients), food (candy, stabilizing agents) and other commercial products (cosmetic industry, filter membranes…) [29]. Films from bacterial cellulose are mostly made by a drying a gel in a flat surface like glass plate in air. The results from the researches has shown that this type of films differ from the conditions they were prepared. There was not a lot of research regarding bacterial cellulose and coating on the paper. There are researches who made studies with mentioned polymer as mixture with cotton pulp and bacterial cellulose [30]. The results have shown improved tensile properties and folding endurance has increased. The cause of improved properties are bacterial cellulose fragments, which are entangled on the surface and bind the fibres. In this case, coating on paper could be improved as sizing agents, because of its great capability of binding with fibres. The use of bacterial cellulose is promising as coatings for durable papers or high filler content papers like bank notes, bible papers [26]. On the other hand it can be used as improvement of other papers or restoration, repairs of old documents [31].

3.3. Xanthan

Xanthan is polysaccharide (Figure **4**), produced from bacteria *Xanthomonas campestris* [3]. Its use is manly focused in food industry as food additive, in cosmetic industry as an ingredient to prevent separating components in the product. It consists of pentasaccharide units, comprising glucose, mannose and glucoronic acid [32]. The production of Xanthan consists of fermentation of glucose, lactose and sucrose. After the fermentation it is precipitated from the growth medium with isopropyl alcohol. The last step in the production is drying, where the xanthan is produced to powder. The main problem of xanthan powder is mixing in a hot or cold water, because it has a tendency to form lumps.

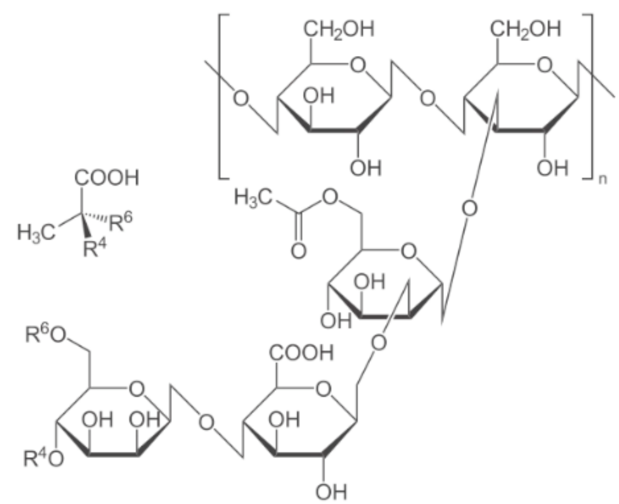

Figure 4: Chemical structure of Xanthan [32].

There has been many research done on this topic and the solutions were [33, 34]:

- Xanthan could be premixed with other powder substances if needed,
- Slow addition of the powder into the water, vortex,
- If making xanthan gum, where oils and alcohol are needed, then it should be mixed firstly with them and last with water.

Several works has been made on modification of calcium carbonate and use of organic substances such as xanthan, starch, cellulose derivate, as fillers in papermaking [35]. Xanthan is mainly used as a filler in papermaking, but less as surface coating.

3.4. Curdlan

The production of Curdlan is oriented from non-pathogenic bacteria named *Alcaligenes faecalis* and mostly has been used in the production of gels in food industry and also in pharmacy [4]. It consists of linear beta -1, 3-linked glucose as seen in Figure **5** [36]. For Curdlan it is known that is insoluble in water. Because of its heat-gelling and water-binding functionalities is very important in the food industry [37]. Less is used in papermaking and coating industry; there are only experiments in the laboratory scales. Curdlan attracts the interest as biopolymer in many fields, it could be successfully used in packaging industry due to excellent film forming ability, non-toxicity, biodegradability and relative abundance. Due to its great water insoluble and thermal characteristics, it could be used to improve a water barrier capacity and the thermal stability of bilayer and multilayer packaging films. The fabrication of blend films *via* casting method could one of the most appropriate methods.

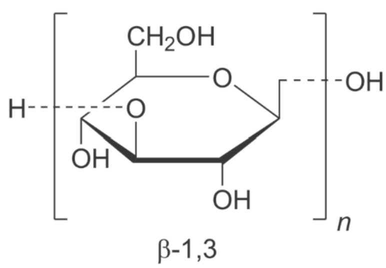

Figure 5: Chemical structure of Curdlan [36].

3.5. Pullulan

Pullulan is produced from microbe named *Aureobasidium pullalans*, which is less soluble in water. His properties could be used as an advantage for coatings on packaging materials, where water resistance or water and oxygen barrier is the main property [5]. It is a water soluble glucan gum, which is produced by grooving a yeast like fungus *Aureobasidium pullalans* [38]. Pullulan consists of glucose and its often described as α-(1→6) linked polymer of maltrose subunit, as seen in Figure **6** [39]. Because of great adhesive properties, it can be used to form fibres and strong films, with very low oxygen permeability, high heat sealable properties and good printing qualities. In comparison with other polymers (for example starch), which can be used in paper and packaging industry, it has higher adhesive strength. There has been hundreds of patents, but mostly in the food and pharmaceutical industry. Pullulan can be chemically derivatized in order to change water solubility or provide reactive groups [40]. The occurrence of α-(1→6) linkages in pullulan is responsible for flexible structure and its solubility. Films made from pullulan are thin (5-60µm), have smooth surface, are clear, have high oxygen permeability and excellent mechanical properties [40, 41]. In packaging, such films can also have added flavours and colour. Pullulan is unique polymer, which could be widely used in many fields of industry and technology. The main problem of its larger use is price, because in comparison to other polymers from microorganisms is quite expensive, according to data in the market it's three times higher than other polysaccharides (xanthan). With technical improvements, production optimisation, pullulan could be used in larger scale as coating and packaging material for food, powdered coffee and tea etc. The products could be easily decomposed, because they don't contaminate the environment.

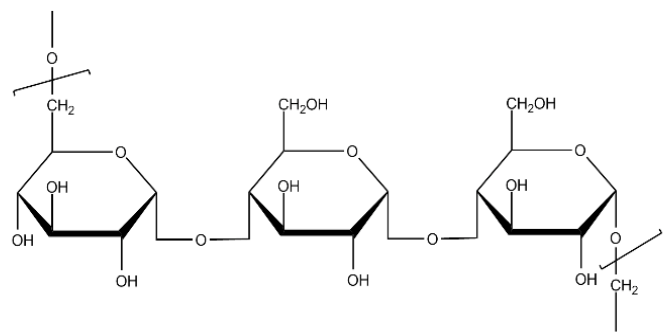

Figure 6: Chemical structure of Pullulan [39].

3.6. Microbial Hyaluronic Acid

Hyaluronic acid (HA) is a natural polymer, composed of disaccharide repeats of *D*-glucuronic acid (GlcUA) and *N*-acetylglucosamine (GlcNAc) joined alternately by β-1, 3 and β-1, 4 glycosidic bonds (Figure **7**) [42, 43]. The molecular weights of HA from different sources are highly variable, ranging from 10^4 to10^7Da. Commercially it is available and valuable medical product. In human body is present in the skin (dermis and epidermis), almost 50% and about 35% in skeleton and muscles. Traditionally it was extracted from rooster combs. First microbial HA was produced *via* microbial fermentation from *Streptococcus* sp., lately from *S. zooepidemicus and Bacillus subtilis* [44]. In the field of packaging HA is still not present. There is research with coating the nanofibres poly(D, L-lactide) in order to produce medical implants and to prevent secondary infections [44]. Since HA has a medical background, there should be many research done, to determine the effect and application possibilities in packaging industry.

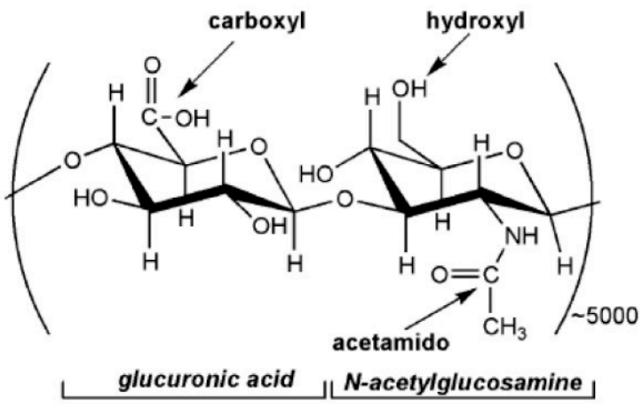

Figure 7: Chemical structure of Hyaluronic acid [43].

3.7. Poly-γ-Glutamic Acid (γ-PGA)

Poly-γ-glutamic acid (γ-PGA) is naturally occurring biopolymer, composed of repeating unites of l-glutamic acid, d-glutamic acid or both, as seen in Figure **8** [45]. Is a bacterium synthesized polymer from *Bacillus licheformis*.

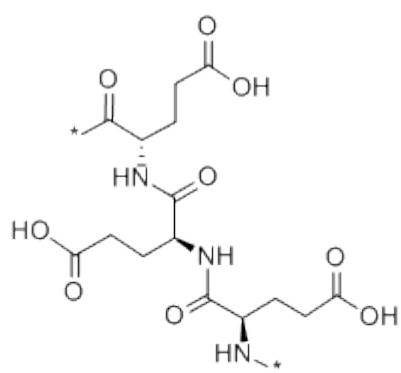

Figure 8: Chemical structure of Poly-γ-glutamic acid [45].

The development of innovative biomedical materials and approaches require methodological simple and cheap production method [46]. γ-PGA is, as HA, mostly used in medicine and biotechnology. Due to its different properties such as conformational states, molecular mass, enantiomeric properties, biodegradability, non-toxicity, it has been successfully used in medical and food industry, as well as in wastewater industry. Among other novel applications it has the potential in many applications in material science. γ-PGA and its derivatives offer a wide range of applications. The applications are summarized in Table **1** [47-54]. In material production, mostly used applications are in fibres coating. From literature review it could be seen that most of the research and applications in the paper industry are in the field of paper production (as dispergents). γ-PGA could also be used as antimicrobial, thermoplastic material, film, which could be used as very effective packaging material [48-54]. γ-PGA is still in the invention process regarding packaging industry as well as paper industry. Coating with such polymers offer many advantages and on the other hand great caution to control possible premature degradation should be done.

Table 1: Industrial Applications of Poly-γ-Glutamic Acid

Application		Function	Reference
Medical	Biological adhesive, medical bonding knit, drug carrier, medical healing material	Gene therapy, cancer drug	45, 46, 47, 49, 52
Food industry	Thickener	Viscosity enhancement for beverages	45, 46, 49, 50, 51
	Aging inhibitor or texture enhancer	Bakery products for the prevention of aging, improvement of textures	45, 48, 50, 51
	Bitterness relieving agents	Relief of bitter taste	45, 48, 50, 51
	Feed additives for animals	Increases minerals absorption and egg-shells strength; decreases body fat, etc.	45, 48, 50, 51
Cosmetics	Hummectant	Reducing the loss of moisture in skin lotions; reducing the moisture in food as food additive	45, 53
Materials	Dispersing agent	Used in paper making and production	45, 53
	Biodegradable packaging material	Thermoplastic material	48, 54
	Coating fibres	For medical use	49
Others	Membrane absorbent	Heavy metals pick-up	45, 48
	Water absorbent	Absorbs water in diapers	45, 47, 48
	Dispersant	Dispersing pigment and minerals in detergent and cosmetics	45, 47, 48

3.8. Microbial Exopolysaccharides

Microbial exopolysaccharides (EPS) are produced by microorganisms and present unused field, especially in material science and only few have been industrially developed. Few microorganisms can produce over 40gL^{-1} in costly production [55]. The main advantage of EPS is that they may either be homopolymeric or heteropolymeric in composition, of diverse high molecular weights (10–1000 kDa) [56]. The researches and discoveries of different types of EPS has been in recent years detected. One of the limitations are costs of the production. Many bacteria can synthesise EPS with commercially properties and applications. Mostly are used in therapeutic applications and numerous health benefits (antitumor effects, antioxidant effect, ability to lower the blood cholesterol etc.). In the food and dairy industry EPS is used as thickener, which provides emulsifying activity [57]. The biosynthesis occurs in different growth phases and depends on environmental conditions. The organism used in the production has also an influence on biosynthesis of EPS. The synthesis involves large number of genes coding for enzymes and proteins. Due to natural existing mechanisms in microorganisms, it is often necessary to resort to physical or chemical extraction methods to improve the yield of EPSs at an industrial level. In the packaging field, EPS has been used as biofilm for food products in the context of food hygiene [58]. There has been also developed anti-corrosion coating using EPS on corrosion sensitive metal and stainless steel [59]. The future prospects of EPS in coating industry is in development the coating, antimicrobial surfaces for food packaging. Nevertheless it is important to select the appropriate composition of EPS.

4. CONCLUSIONS AND FUTURE PROSPECTS

For the most packaging applications water vapour and oxygen permeability are required to protect the product. In the future edible coatings are very promising for improving the quality of packaging. Edible films and coatings which are discussed in this article are biodegradable and perfectly safe for the environment. On the other hand, the price for polymers which are produced from microorganisms are still quite high, but the used quantities are very low and applied for specific surface and functionalities. Aspects that should be taken under consideration of the production of these polymers in the future are to lower the production costs (especially PHA) and find added values for certain coating and substrate. Furthermore even more studies about the interactions between the coatings, substrates, active compounds and microorganisms should be done in the future.

Discussed polymers in the research are hydrocolloids, whose rheological properties play the key role in applications in coating industry. Their behaviour can be attributed to the presence of hydroxyl groups in their structure. This leads to their H-bonding interactions in the aqueous system [42]. The production and application of polymers, produced from microorganisms requires interdisciplinary knowledge.

The literature survey shows that edible coatings have the potential to serve the green image of packaging applications and to replace the fossil-oil based polymers. Coatings with such polymers give a paper recyclability, biodegradation and certain improved functionalities. From the presented review an appropriate combination of bio-based polymers and substrates (paper, cardboard) has the potential to provide new, highly added value and protective coating for paper and packaging.

However, there is still a need for further investigations and studies for specific products and problems that can occur on the packed product and on the packaging itself.

Genetic controls of polymer production from microorganisms offer many advantages in tailoring biopolymer structure, function and end product properties. Nevertheless at the end, it is important to control the environment and to prevent possible premature degradation of the polymers.

CONFLICT OF INTEREST

The author declare no conflict of interest.

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