



HAL
open science

Can synthetic biology really empower microbial biopolymers as efficient food contact materials?

Muhammad Rehan Khan, Elena Torrieri, Florent Allais, Sami Fadlallah

► To cite this version:

Muhammad Rehan Khan, Elena Torrieri, Florent Allais, Sami Fadlallah. Can synthetic biology really empower microbial biopolymers as efficient food contact materials?. Trends in Food Science and Technology, 2024, 143, pp.104250. 10.1016/j.tifs.2023.104250 . hal-04651997

HAL Id: hal-04651997

<https://agroparistech.hal.science/hal-04651997v1>

Submitted on 17 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Can synthetic biology really empower microbial biopolymers as efficient food contact**
2 **materials?**

3 Muhammad Rehan Khan^{a*}, Elena Torrieri^a, Florent Allais^b, and Sami Fadlallah^{b*}

4 ^aDepartment of Agricultural Science, University of Naples Federico II, Portici (NA), Italy.

5 ^bURD ABI (Agro-Biotechnologies Industrielles), CEBB, AgroParisTech, Pomacle, 51110,
6 France.

7 **Correspondence:** rehankhan.unina@outlook.com; sami.fadlallah@agroparistech.fr

8

9

10

11

12

13

14

15

16

17

18

19

20

21 **Abstract**

22 This opinion paper explores the potential of integrating synthetic biology into microbial
23 polymers to produce tailored biopolymers for food packaging applications. Synthetic biology
24 has shown precise control over metabolic machinery, enabling the manipulation of pathways
25 involved in microbial biopolymer production. However, there is limited literature available
26 on utilizing the same pathways for designing tailored biopolymers suitable as efficient food
27 contact materials. This is primarily due to the regulatory status of microbial polymers as
28 determined by food safety authorities. One possible solution is to leverage synthetic biology
29 tools by adopting safety assessment protocols established within the regulatory framework.
30 By considering the advantages of synthetic biology-driven microbial polymers, this
31 innovative approach has the potential, not only to replace conventional methods but also to
32 provide additional value by addressing environmental concerns associated with traditional
33 food packaging.

34 **Keywords:** synthetic biology; tailored biopolymers; microbial polymers; food packaging;
35 microbial cell factories

36

37

38

39

40

41

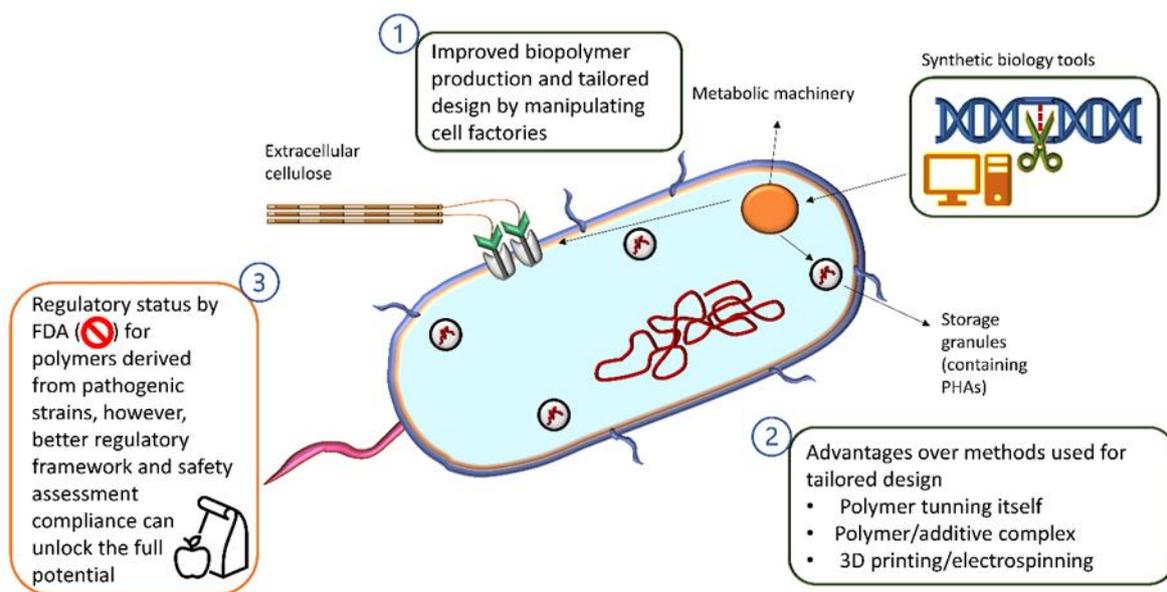
42

43 **1. Introduction**

44 Recently, there has been a growing interest among researchers in exploring biopolymers as
45 green and sustainable alternatives to synthetic materials for the development of active
46 packaging (Sid et al., 2021). Moreover, in the past decade, considerable research efforts have
47 been dedicated to utilizing microbial sources to obtain biobased materials, thanks to their
48 biocompatibility, biodegradability, and excellent barrier properties stemming from their
49 inherent hydrophobic nature (Salgado et al., 2021). However, their use in food packaging was
50 limited due to their poor thermo-mechanical properties (Battezzore et al., 2019). To address
51 this limitation, the concept of polymer tunability was introduced, enabling the production of
52 polymers with tailored design for specific applications. This involves modifying the chemical
53 structure of the polymer (Hazer and Steinbüchel, 2007), incorporating additives into the
54 polymer matrix (Battezzore et al., 2019; Carboué et al., 2021; Khan et al., 2023), or using
55 new technologies such as electrospinning or 3D printing to develop tailored polymers (Zhao
56 et al., 2021; Zieliński et al., 2023). Still, these methodologies face challenges such as
57 precision control and scalability (NRC, 2015). This is where synthetic biology comes into
58 play.

59 Synthetic biology is a multidisciplinary field that offers a powerful toolset to design new
60 biological systems with enhanced functionalities. Notably, synthetic biology tools such as
61 CRISPR-cas9 (a gene-editing tool), promoter engineering, and metabolic modeling, have
62 been effectively used to improve the production of microbial polymers for biomedical
63 applications (Katz et al., 2018). These approaches can also be employed to develop tailored
64 biopolymers with enhanced functionalities to cater to the needs of the food packaging
65 industry. Several strategies have been used to produce higher amounts of polymer from
66 microbial sources, such as modifying metabolic pathways and altering the PHA
67 (Polyhydroxyalkanoates) synthase enzyme (Gahlawat et al., 2020). Similar strategies were

68 explored to improve thermo-mechanical properties by manipulating *phaC* gene sequence to
 69 achieve desired expression for polymerization (Tanadchangsang and Yu, 2012). Despite their
 70 many advantages i.e., sustainable production, tailored properties, and cost-effectiveness the
 71 regulatory status of certain microbial polymers (e.g., alginates and exopolysaccharides from
 72 gram-negative bacteria) derived from synthetic biology is a major hurdle in using these
 73 materials as active packaging (Hinchliffe et al., 2021) (Fig 1). Thus, the aim of this opinion
 74 herein is to answer the critical question: Can synthetic biology really empower microbial
 75 biopolymers as efficient food contact materials by controlling polymer tunability?



76
77

78 **Figure 1.** Manipulation of microbial cell factories for tailored biopolymer production and its
 79 status.

80 **2. Using synthetic biology as a tool for microbial biopolymers production**

81 Recently, synthetic biologists have embraced genetic engineering approaches such as
 82 modulating DNA fragments and employing computational models to engineer living
 83 organisms efficiently (Tang et al., 2021). This involves controlling microbial cellular
 84 behavior by using advanced synthetic biology methods, including the construction of

100 **Figure 2.** Synthesis of some novel PHAs i.e., P(3HB-*co*-4HB) and P(3HB-*co*-3HV)
101 polymers with *phaC* as a key player in their production (adopted from Lv et al., 2015 and
102 Chen et al., 2019).

103 *2.1 Regulatory pathways for controlling biosynthesis of microbial biopolymers*

104 The biosynthesis of biopolymers such as cellulose, alginate, polyphosphate, and PHAs in
105 microbes is controlled by regulatory pathways that facilitate their production and response to
106 environmental signals. These pathways regulate gene expression through transcription level
107 (conversion of DNA to RNA) and post-translational level (modification occurring after
108 protein synthesis) modifications. Microbes can precisely control the synthesis of biopolymers
109 in response to changes in their environments. Transcription factors activate promoters that
110 influence the expression of genes responsible for the bioproduction of functional materials
111 such as P(3HB) and P(3HB-*co*-4HB) (Babele et al., 2019). By controlling the expression of
112 these genes or providing a specific external stimulus (temperature-sensitive
113 promoters/circuits, chemical inducers, pH levels, nutrient availability, light, and quorum
114 sensing) we can exercise control over biomaterial production (Fig 2).

115 While the mechanism of bacterial exopolysaccharides, a key component of virulence, is
116 extensively studied, limited information is available on using similar pathways to obtain
117 functional biomaterial from non-pathogenic species (Hay et al., 2014). Several proof-of-
118 concept studies have demonstrated the potential of genetic engineering approaches to
119 enhance biopolymer production. Another example of using a combination of genetic
120 engineering approaches to get enhanced biopolymer production was reported by Dumon et al.
121 (2001). The authors enhanced fucosylated oligosaccharide production by redirecting cellular
122 carbon and energy influx towards biomaterial production, rather than biomass/metabolic
123 byproducts. This was achieved by identifying and downregulating competing pathways that

124 led to the formation of unwanted byproducts. Sim et al. (1997) reported a reduced molecular
125 weight (1.3×10^6 Da) for the produced polyhydroxy butyrate (PHB) polymer by genetically
126 modified *E. coli* in comparison to the native *E. coli* (4.0×10^6 Da) by modulating PHA
127 synthase activity which indeed can impact the structural and mechanical properties of the
128 polymeric materials (Tanadchangsaeng and Yu, 2012). Despite the potential of utilizing
129 microbial biopolymers as food contact materials, exploration of these avenues remains
130 limited in literature.

131 *2.2 Microbial self-assembly structures as packaging material*

132 Bacterial self-assembly structures (e.g., fimbriae, pili, and flagella) are used for adhesion in
133 response to chemical and physical stimuli especially during pathogenesis, due to this unique
134 property they hold promise for producing nanomaterials for their application in packaging.
135 The precise arrangement of protein building blocks in self-assembling structures such as
136 flagella, high surface area to volume ratio, and their polymorphic nature can be useful in the
137 production of nanostructures for novel food packaging. These nanostructures can transform
138 the polymer matrix in such a way that they restrict polymer chain mobility, thereby
139 improving tensile properties. Additionally, they create convoluted pathways for gas/water
140 molecule transport and improve the barrier properties of the packaging material (Abbineni
141 and Mao, 2010; Bera et al., 2019). Chen et al. (2014) reported a dual-functioning *Escherichia*
142 *coli* strain capable of acting as a material synthesis platform and producing functional
143 material i.e., amyloid. This work prompted further investigations, including the improvement
144 of the tunability of the above system by using three-dimensional (3D) printing (Cao et al.,
145 2017). The utilization of 3D patterned curli fibrils has demonstrated the capability to
146 assemble inorganic materials upon external stimuli, resulting in the development of structural
147 materials with well-defined physiochemical properties.

148 **Table 1.** Comparison of methods available for tailored biopolymer design.

Method	Pros	Cons	Challenges	References
Synthetic biology	<ul style="list-style-type: none"> - Precise control over material production and properties. - Utilizing renewable resources and reducing environmental impact. - Cost-effective once established. - Biodegradation control. 	<ul style="list-style-type: none"> - Long developmental phase for strain engineering and its optimization. 	<ul style="list-style-type: none"> -Regulatory constraints 	Anderson et al., 2018
Chemical modification	<ul style="list-style-type: none"> - Using simple chemical reactions to make tailored biopolymers. - Availability of (green) chemical modification techniques. 	<ul style="list-style-type: none"> - Chemical reactions may use toxic, non-food grade materials. - Loss of biopolymer inherent property. 	<ul style="list-style-type: none"> - Impact on biocompatibility and biodegradability. 	El Itawi et al., 2022; Fadlallah et al., 2021; Wang et al., 2015
Using additives	<ul style="list-style-type: none"> - Easy processing method and incorporation for desired properties. - Wide range of additives catering to fine-tuning the material properties. - Modulating material properties based on additive concentration. 	<ul style="list-style-type: none"> - Limited control over additive distribution in the polymer matrix. - Migration of additives into foods. - Compatibility issues between polymer and additive. 	<ul style="list-style-type: none"> - Additive homogeneity and stability. 	Samyn and Taheri, 2020
3D printing	<ul style="list-style-type: none"> - Reduced material waste due to precision. - Manufacturing flexibility. 	<ul style="list-style-type: none"> - Difficult optimization of parameters for a specific polymer. - High cost. 	<ul style="list-style-type: none"> - Inconsistent mechanical properties. 	
Electrospinning	<ul style="list-style-type: none"> - Good for producing nanomaterials with high surface area. - Enhanced barrier and mechanical properties. 	<ul style="list-style-type: none"> - Complex setup and control over method is difficult. - Difficulty in handling nanofibers and mats. 	<ul style="list-style-type: none"> - Scaling up at industrial level. 	Romero-Araya et al., 2021

149

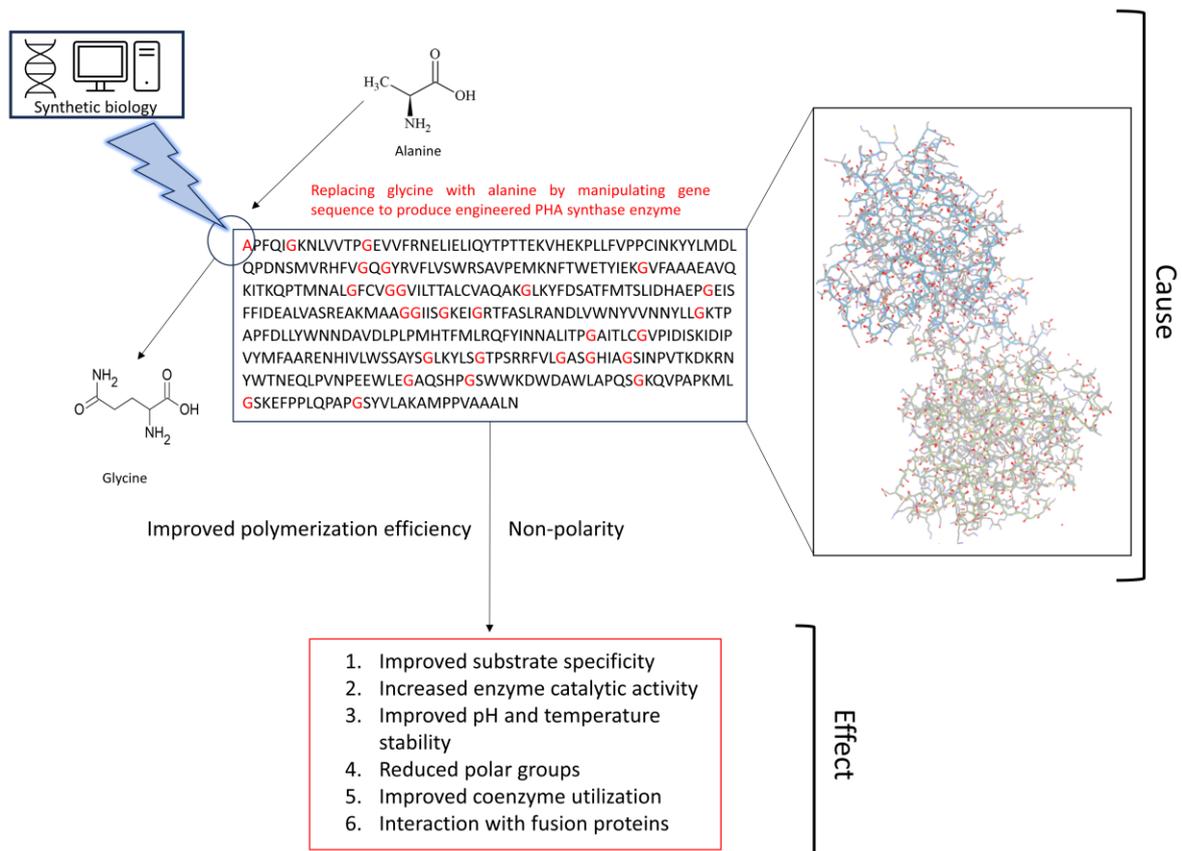
150 **3. Tailored biopolymer design for sustainable food packaging**

151 Within the existing literature, three main methodologies have been employed to produce
152 tailored biopolymers for food packaging applications. These methods involve: (1) modifying
153 the polymer itself by chemical modifications such as esterification, etherification,
154 transesterification, ring-opening polymerization, and oxidation, (2) incorporating additives
155 such as antioxidants, antimicrobials, or plasticizers through casting or extrusion processes,
156 and (3) using techniques such as 3D printing by creating 3-dimensional objects by depositing
157 successive polymer layer to have more control on structure, and electrospinning which aligns
158 polymer chains along fiber axis (Fraeye et al., 2010; Ghosal et al., 2018; Munteanu and
159 Vasilie, 2019; Shahbazi and Jäger, 2020). Still, these approaches have several
160 limitations/disadvantages when compared to synthetic precision biology (Table 1). For
161 instance, synthetic biology approaches allow precise and targeted modifications of the
162 microbes at the genetic level to produce polymers with tunable properties. Genetic
163 engineering tools to precisely engineer the genetic makeup of the microorganism in such a
164 way that not only optimizes production but also enhance physicochemical properties of the
165 resultant materials since that is not easily achievable by tuning methods i.e., 3D printing and
166 electrospinning. Synthetic biology can provide a high level of cost-effectiveness,
167 customization, and adaptability to produce tailored biopolymers for scalable production. For
168 instance, synthetic biology has been shown to affect the microstructure of bacterial
169 cellulose/pullulan composites by decreasing the space between polymer fibers due to
170 hydrogen bonding between polar groups of cellulose and pullulan which led to significantly
171 higher tensile properties (tensile strength: 27 MPa) as compared to control cellulose materials
172 (16 MPa) (Zhantlessova et al., 2022). Even though the authors did not observe the barrier
173 properties of these materials, microstructure analysis indicates possibly higher barrier
174 characteristics of these composite polymer materials than cellulose. Fang et al. (2015)
175 similarly produced a cellulose copolymer by using genetic engineering and highlighted that

176 cellulose copolymer had a more compact structure than native cellulose which also impacted
177 the surface hydrophobicity of the materials. The contact angle rose significantly from 25° to
178 46° for the cellulose copolymer which indicates that cellulose nanofibers can prevent water
179 permeation and can act as a food packaging material. Recently, Gahlawat et al. (2020)
180 adequately highlighted the importance of using metabolic engineering strategies, i.e.,
181 CRISPR/Cas9 and ribosome-binding site optimization, for high-output and cost-effective
182 production of PHAs. However, limited attention has been given to modifying the mechanical
183 and barrier properties of these polymers for food contact applications. Moreover, Li et al.
184 (2017) recently targeted *phaC* gene to get improved PHA production from the over-
185 expression of PHA synthase enzyme, however, no attention was given to utilizing *phaC* gene
186 to get tailored polymer with desired properties. To address this, we suggest altering specific
187 amino acid sequences (i.e., replacing glycine with alanine) to get increased polymerization
188 efficiency and non-polarity or introducing additional motifs and domains such as self-
189 assembly peptides and elastin into PHA synthase which can improve the mechanical and
190 thermal properties of the films (Fig 3) (Singh et al., 2015). Altering amino acid sequence can
191 broaden the substrate acceptance range of PHA synthase enzyme which can lead to improved
192 polymerization efficiency, similar trends can also be observed for improved catalytic activity
193 of the enzyme due to mutation. On the other hand, replacing glycine with alanine in the PHA
194 synthase enzyme can lead to a change in the stereochemistry of the polymer thus affecting
195 polymer's properties and crystallinity. Furthermore, altering the substrate specificity of PHA
196 synthase enzyme can redirect the polymerization process towards different monomer
197 substrates, resulting in PHA-based polymers with improved barrier characteristics for food
198 packaging applications. Similar concepts can also be applied to other genes, such as *phaZ*,
199 which encodes a depolymerization enzyme. By controlling this gene, we can control the
200 polymer degradation rate, however, this requirement is product-specific as far as packaging is

201 concerned (Santos-Beneit et al., 2023). For instance, the gene encoding for *phaZ* enzyme can
 202 be controlled by using promoters that can respond to specific environmental factors i.e., pH.
 203 A pH-dependent promoter can upregulate *phaZ* gene expression when the pH of the
 204 surrounding environment or product falls below a certain range. This can allow a pH-
 205 dependent polymer degradation. This phenomenon can also be used for the release of
 206 bioactives from the packaging material when the product displays a pH comparable to
 207 spoilage conditions, however, in this scenario downregulation of the gene should be
 208 considered.

209



210

211 **Figure 3.** Scheme for the increased polymerization efficiency and non-polarity by altering the
 212 amino acid sequence in PHA synthase.

213 *Potential as food contact materials*

214 As discussed in previous sections, limited literature is available on the utilization of microbial
215 biopolymers obtained from synthetic biology as efficient food contact materials. Thus, here
216 we briefly discuss how different additives were used in the literature to modify the structure
217 of microbial polymers in such a way that their properties (barrier and mechanical) relevant to
218 food applications were significantly improved. For instance, the addition of essential oils into
219 PHB matrices could lead to reduced interactions between polymer chains; as a result, strong
220 polymer-polymer interactions could be replaced by weaker polymer-oil interactions in the
221 intermolecular film network which could lead to 10-30% decrease in tensile strength (TS)
222 and up to 100-200% improvement in elongation at break (EAB) (Mittal et al., 2023; Rech et
223 al., 2021). Contrarily, the addition of nanoparticles (NPs) into the PHA blends could lead to
224 improved tensile properties of the films (up to a certain extent) due to uniform dispersion of
225 large surface area NPs which enhanced interfacial adherence and crystallinity between
226 polymer phases through hydrogen bonding (Lan and Sun, 2018; Mittal et al., 2023). On the
227 other hand, the water vapor permeability (WVP) of the microbial biopolymers was also
228 affected by the type of additives used. For instance, the addition of oils into the PHA matrix
229 led to a 225% increase in the WVP of the packaging films (Castro-Mayorga et al., 2017;
230 Mittal et al., 2023). The hydrogen bond formation between polymer hydroxyl groups and
231 oxygen atoms of the NPs has been shown to improve the permeability properties (~40%) of
232 the materials as compared to the neat ones (Venezia et al., 2023). These properties are
233 essential to utilize microbial biopolymers as food contact materials, for instance, PHB,
234 PHBV, P(3HB-CO-4HB), microalgal exopolysaccharides, riclin, and bacterial cellulose have
235 been utilized to pack a variety of food products like white bread, cheese, shrimp, and fruits
236 and vegetables, however, their application of these materials have only been limited to keep
237 the microbial burden below ($< 6 \text{ Log CFU/g}$) and to serve as a colorimetric indicator of
238 spoilage as shown in Table 2. More studies needs to be conducted by utilizing synthetic

239 biology to develop food contact materials since most of the existing studies needed
 240 polymer/additive technology to fine tune polymers according to product requirements.

241 **Table 2.** Application of microbial biopolymers on food products

Packaging composition	Mechanical properties	Barrier properties	Food application	References
PHB, nanosilica, and clove oil	TS: 6.5 to 17.3 Mpa	WVP: 0.30-0.69 g.mm/m ² .kPa.day	Brown bread	Mittal et al., 2023
PHB/PCL, organoclay, and nisin	TS: 6.29-7.49 MPa; EAB: 0.72-3.03 %	WVP: 1.05-2.62×10 ⁻¹¹ g.s ⁻¹ .m ⁻¹ .Pa ⁻¹	Ham	Correa et al., 2017
Poly (3-hydroxybutyrate-co-4-hydroxybutyrate) and thyme oil	TS: 51.8-87.4 MPa EAB: 28.1-40.1 %	WVP: 0.31-0.42 g.mm/m ² .kPa.day	White bread	Sharma et al., 2022
PHBV, eugenol, and carvacrol	NA	NA	Cheese, chicken breast, pumpkin, and melon	Requena et al., 2019
Riclin and PVA	TS: 25-60 MPa EAB: 5-60 %	WVP: 1.1-2 ×10 ⁻¹¹ g/(m.s.Pa)	Shrimp	Miao et al., 2023
Bacterial cellulose and silver NPs	TS: 17-26 MPa EAB: 4.77-6.18 %	NA	Oranges and tomatoes	Atta et al., 2021

Bacterial cellulose, pomegranate peel extract, green tea extract, and rosemary extract	NA	NA	Button mushroom	Moradian et al., 2018
--	----	----	-----------------	-----------------------

242 Whereas NA= not applicable

243 **4. Limitations and future directions**

244 McCarty and Ledesma (2019) argued that the utilization of synthetic biology in microbial
245 consortia can provide distinct advantages over monocultures in terms of enhanced production
246 of microbial biopolymers (exopolysaccharides) with tunable functional properties. These
247 properties encompass antioxidant and antibacterial properties as microbial consortia can also
248 release active metabolites (pyocyanin, lactic acid, and other bacteriocins) (Fouillaud and
249 Dufossé, 2022). This advantage stems from the labor division between different species
250 within the consortium, which allows for a shared metabolic burden and broader metabolic
251 capabilities. While this is an interesting prospect, the use of synthetic microbial consortia has
252 inherent limitations. These include the constraint of a limited number of genetically
253 engineered species that can be effectively incorporated into the consortium, the complexity
254 associated with designing and engineering microbial consortia, and their sensitivity to
255 environmental changes which can disrupt the delicate balance within the microbial consortia.
256 Concerns regarding GMOs (Genetically Modified Organisms) were also raised by the EU and
257 the USA in the past two decades which can limit the usage of synthetic biology in fine-tuning
258 microbial polymers. Still, the US GMO approval process is less rigorous than the EU and is
259 voluntary in nature. Although, the FDA encourages developers to submit their GMO-based
260 products for pre-market clearance, however, it's not mandatory, while the EU has more

261 stringent labeling requirements even if the product contains more than 0.9% GMO content
262 (Anderson and Jackson, 2003; Eriksson et al., 2018). This could be a hurdle in utilizing
263 synthetic biology for developing tunable packaging materials for food contact applications.
264 However, this problem can be resolved by adopting robust safety assessment protocols
265 implemented by manufacturers during microbial polymer production and tuning steps,
266 combined with the establishment of a clear and uniform regulatory framework by relevant
267 authorities. Nonetheless, synthetic biology can provide a way forward to develop smart
268 packaging materials with tunable properties in which materials can respond to changes in the
269 food environment (i.e., temperature, pH, and or freshness). For instance, intermolecular
270 bonding between polymeric chains and bioactive can be utilized to effectively release
271 compounds from packaging into the food due to the pH sensitivity of the intermolecular
272 bonding as displayed by Zhou et al. (2016).

273 **5. Conclusions**

274 Despite their biodegradable and biocompatible nature, some microbial-based polymers like
275 PHAs suffer from poor thermo-mechanical properties due to their highly crystalline structure.
276 This issue can be effectively addressed by leveraging cutting-edge genetic engineering tools
277 to tune their properties specifically for food packaging applications. This approach offers an
278 alternative to costly and traditional modifying the polymer itself, adding additives into the
279 mixture, or using methods such as electrospinning and 3D printing. While the potential of
280 using genetic engineering to produce tailored biopolymers is an un-tapped gold mine, there
281 are some regulatory considerations to overcome. Currently, some microbial-based polymers
282 may not have been Generally Recognized as Safe (GRAS) by the FDA (which mainly
283 depends on the status of the production strain). These factors can limit the full utilization of
284 synthetic biology to enhance microbial biopolymers for innovative food packaging. We
285 believe that adopting robust safety assessments implemented by manufacturers during

286 microbial polymer production (by using advanced chromatographic techniques), combined
287 with the establishment of a clear and uniform regulatory framework by relevant authorities,
288 can address these concerns. This approach will enable the realization of the full potential of
289 using GRAS microbial cell factories as valuable sources for tailored biopolymers, ensuring
290 their safe and innovative utilization in the food packaging industry.

291 **Conflicts of interest**

292 The authors have no conflicts of interest.

293 **Author contributions**

294 Conceptualization, data curation, writing-original draft, writing-review and editing: MRK;
295 Conceptualization, management, supervision, writing-original draft, writing-review and
296 editing: SF; Supervision, writing-review and editing: ET and FA.

297 **Acknowledgments**

298 This study was carried out within the Agritech National Research Center (Task 8.1.3.
299 valorization of the waste to obtain biomaterials) and received funding from the European
300 Union via Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA
301 (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032
302 17/06/2022022, CN00000022). This manuscript reflects only the authors' views and
303 opinions, and neither the European Union nor the European Commission can be considered
304 responsible for them.

305 **References**

306 Abbineni, G., & Mao, C. (2010). Design and applications of genetically engineered
307 nanocomposites. Wiley-VCH.

308 An, B., Wang, Y., Huang, Y., Wang, X., Liu, Y., Xun, D., ... & Zhong, C. (2022). Engineered
309 living materials for sustainability. *Chemical Reviews*, 123(5), 2349-2419.

310 Anderson, K., & Jackson, L. A. (2003). Why are US and EU policies toward GMOs so
311 different?.

312 Anderson, L. A., Islam, M. A., & Prather, K. L. (2018). Synthetic biology strategies for
313 improving microbial synthesis of “green” biopolymers. *Journal of biological chemistry*,
314 293(14), 5053-5061.

315 Atta, O. M., Manan, S., Ul-Islam, M., Ahmed, A. A. Q., Ullah, M. W., & Yang, G. (2021).
316 Silver decorated bacterial cellulose nanocomposites as antimicrobial food packaging
317 materials. *ES Food & Agroforestry*, 6, 12-26.

318 Babel, P. K., Kumar, J., & Chaturvedi, V. (2019). Proteomic de-regulation in cyanobacteria
319 in response to abiotic stresses. *Frontiers in Microbiology*, 10, 1315.

320 Battezzato, D., Noori, A., & Frache, A. (2019). Hemp hurd and alfalfa as particle filler to
321 improve the thermo- mechanical and fire retardant properties of poly
322 (3- hydroxybutyrate- co- 3- hydroxyhexanoate). *Polymer composites*, 40(9), 3429-3437.

323 Bera, S., Mondal, S., Xue, B., Shimon, L. J., Cao, Y., & Gazit, E. (2019). Rigid helical-like
324 assemblies from a self-aggregating tripeptide. *Nature Materials*, 18(5), 503-509.

325 Cao, Y., Feng, Y., Ryser, M. D., Zhu, K., Herschlag, G., Cao, C., ... & You, L. (2017).
326 Programmable assembly of pressure sensors using pattern-forming bacteria. *Nature*
327 *biotechnology*, 35(11), 1087-1093.

328 Carboué, Q., Fadlallah, S., Werghi, Y., Longé, L., Gallos, A., Allais, F., & Lopez, M. (2022).
329 Impact of Bis-O-dihydroferuloyl-1, 4-butanediol Content on the Chemical, Enzymatic and
330 Fungal Degradation Processes of Poly (3-hydroxybutyrate). *Polymers*, 14(8), 1564.

331 Castro-Mayorga, J. L., Fabra, M. J., Pourrahimi, A. M., Olsson, R. T., & Lagaron, J. M.
332 (2017). The impact of zinc oxide particle morphology as an antimicrobial and when
333 incorporated in poly (3-hydroxybutyrate-co-3-hydroxyvalerate) films for food packaging and
334 food contact surfaces applications. *Food and Bioproducts Processing*, 101, 32-44.

335 Chen, A. Y., Deng, Z., Billings, A. N., Seker, U. O., Lu, M. Y., Citorik, R. J., ... & Lu, T. K.
336 (2014). Synthesis and patterning of tunable multiscale materials with engineered cells. *Nature*
337 *materials*, 13(5), 515-523.

338 Chen, Y., Chen, X. Y., Du, H. T., Zhang, X., Ma, Y. M., Chen, J. C., ... & Chen, G. Q. (2019).
339 Chromosome engineering of the TCA cycle in *Halomonas bluephagenesis* for production of
340 copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV). *Metabolic engineering*, 54,
341 69-82.

342 Correa, J. P., Molina, V., Sanchez, M., Kainz, C., Eisenberg, P., & Massani, M. B. (2017).
343 Improving ham shelf life with a polyhydroxybutyrate/polycaprolactone biodegradable film
344 activated with nisin. *Food Packaging and Shelf Life*, 11, 31-39.

345 Dumon, C., Priem, B., Martin, S. L., Heyraud, A., Bosso, C., & Samain, E. (2001). In vivo
346 fucosylation of lacto-N-neotetraose and lacto-N-neohexaose by heterologous expression of
347 *Helicobacter pylori* α -1, 3 fucosyltransferase in engineered *Escherichia coli*. *Glycoconjugate*
348 *journal*, 18, 465-474.

349 El Itawi, H., Fadlallah, S., Allais, F., & Perré, P. (2022). Green assessment of polymer
350 microparticles production processes: a critical review. *Green Chemistry*, 24(11), 4237-4269.

351 Eriksson, D., Harwood, W., Hofvander, P., Jones, H., Rogowsky, P., Stöger, E., & Visser, R.
352 G. (2018). A welcome proposal to amend the GMO legislation of the EU. *Trends in*
353 *biotechnology*, 36(11), 1100-1103.

354 Fadlallah, S., Roy, P. S., Garnier, G., Saito, K., & Allais, F. (2021). Are lignin-derived
355 monomers and polymers truly sustainable? An in-depth green metrics calculations approach.
356 *Green Chemistry*, 23(4), 1495-1535.

357 Fang, J., Kawano, S., Tajima, K., & Kondo, T. (2015). In vivo curdlan/cellulose
358 bionanocomposite synthesis by genetically modified *Gluconacetobacter xylinus*.
359 *Biomacromolecules*, 16(10), 3154-3160.

360 Fouillaud, M., & Dufossé, L. (2022). Microbial secondary metabolism and biotechnology.
361 *Microorganisms*, 10(1), 123.

362 Fraeye, I., Duvetter, T., Doungla, E., Van Loey, A., & Hendrickx, M. (2010). Fine-tuning the
363 properties of pectin–calcium gels by control of pectin fine structure, gel composition and
364 environmental conditions. *Trends in Food Science & Technology*, 21(5), 219-228.

365 Gahlawat, G., Kumari, P., & Bhagat, N. R. (2020). Technological advances in the production
366 of polyhydroxyalkanoate biopolymers. *Current Sustainable/Renewable Energy Reports*, 7,
367 73-83.

368 Ghosal, K., Chandra, A., Roy, S., Agatemor, C., Thomas, S., & Provaznik, I. (2018).
369 Electrospinning over solvent casting: tuning of mechanical properties of membranes.
370 *Scientific reports*, 8(1), 5058.

371 Ghosh, S., Lahiri, D., Nag, M., Dey, A., Sarkar, T., Pathak, S. K., ... & Ray, R. R. (2021).
372 Bacterial biopolymer: Its role in pathogenesis to effective biomaterials. *Polymers*, 13(8),
373 1242.

374 Hay, I. D., Wang, Y., Moradali, M. F., Rehman, Z. U., & Rehm, B. H. (2014). Genetics and
375 regulation of bacterial alginate production. *Environmental microbiology*, 16(10), 2997-3011.

376 Hazer, B., & Steinbüchel, A. (2007). Increased diversification of polyhydroxyalkanoates by
377 modification reactions for industrial and medical applications. *Applied Microbiology and*
378 *Biotechnology*, 74, 1-12.

379 Hinchliffe, J. D., Parassini Madappura, A., Syed Mohamed, S. M. D., & Roy, I. (2021).
380 Biomedical applications of bacteria-derived polymers. *Polymers*, 13(7), 1081.

381 Katz, L., Chen, Y. Y., Gonzalez, R., Peterson, T. C., Zhao, H., & Baltz, R. H. (2018).
382 Synthetic biology advances and applications in the biotechnology industry: a perspective.
383 *Journal of Industrial Microbiology and Biotechnology*, 45(7), 449-461.

384 Khan, M. R., Fadlallah, S., Gallos, A., Flourat, A. L., Torrieri, E., & Allais, F. (2023). Effect
385 of ferulic acid derivative concentration on the release kinetics, antioxidant capacity, and
386 thermal behaviour of different polymeric films. *Food Chemistry*, 410, 135395.

387 Li, D., Lv, L., Chen, J. C., & Chen, G. Q. (2017). Controlling microbial PHB synthesis via
388 CRISPRi. *Applied Microbiology and Biotechnology*, 101, 5861-5867.

389 Lv, L., Ren, Y. L., Chen, J. C., Wu, Q., & Chen, G. Q. (2015). Application of CRISPRi for
390 prokaryotic metabolic engineering involving multiple genes, a case study: controllable P
391 (3HB-co-4HB) biosynthesis. *Metabolic engineering*, 29, 160-168.

392 McCarty, N. S., & Ledesma-Amaro, R. (2019). Synthetic biology tools to engineer microbial
393 communities for biotechnology. *Trends in biotechnology*, 37(2), 181-197.

394 Miao, Y., Chen, Z., Zhang, J., Li, N., Wei, Z., Zhang, Y., ... & Zhang, J. (2023).
395 Exopolysaccharide riclin and anthocyanin-based composite colorimetric indicator film for
396 food freshness monitoring. *Carbohydrate Polymers*, 314, 120882.

397 Mittal, M., Ahuja, S., Yadav, A., & Aggarwal, N. K. (2023). Development of poly
398 (hydroxybutyrate) film incorporated with nano silica and clove essential oil intended for

399 active packaging of brown bread. *International Journal of Biological Macromolecules*, 233,
400 123512.

401 Moradali, M. F., & Rehm, B. H. (2020). Bacterial biopolymers: from pathogenesis to
402 advanced materials. *Nature Reviews Microbiology*, 18(4), 195-210.

403 Moradian, S., Almasi, H., & Moini, S. (2018). Development of bacterial cellulose- based
404 active membranes containing herbal extracts for shelf life extension of button mushrooms
405 (*Agaricus bisporus*). *Journal of Food Processing and Preservation*, 42(3), e13537.

406 Munteanu, S. B., & Vasile, C. (2019). Vegetable additives in food packaging polymeric
407 materials. *Polymers*, 12(1), 28.

408 National Research Council. (2015). *Industrialization of biology: a roadmap to accelerate the*
409 *advanced manufacturing of chemicals*.

410 Rech, C. R., Brabes, K. C., Silva, B. E., Martines, M. A., Silveira, T. F., Alberton, J., ... &
411 Martelli, S. M. (2021). Antimicrobial and physical–mechanical properties of
412 polyhydroxybutyrate edible films containing essential oil mixtures. *Journal of Polymers and*
413 *the Environment*, 29, 1202-1211.

414 Requena, R., Vargas, M., & Chiralt, A. (2019). Eugenol and carvacrol migration from PHBV
415 films and antibacterial action in different food matrices. *Food Chemistry*, 277, 38-45.

416 Romero-Araya, P., Pino, V., Nenen, A., Cárdenas, V., Pavicic, F., Ehrenfeld, P., ... & Flores,
417 M. E. (2021). Combining Materials Obtained by 3D-Printing and Electrospinning from
418 Commercial Polylactide Filament to Produce Biocompatible Composites. *Polymers*, 13(21),
419 3806.

420 Salgado, P. R., D'Amico, D. A., Seoane, I. T., Iglesias Montes, M., Mauri, A. N., & Cyras, V.
421 P. (2021). Improvement of water barrier properties of soybean protein isolate films by poly
422 (3- hydroxybutyrate) thin coating. *Journal of Applied Polymer Science*, 138(5), 49758.

423 Samyn, P., & Taheri, H. (2020). Melt-Processing of Biopolymer Composites with
424 Nanocellulose Additives. In *Advances in Polymer Processing 2020: Proceedings of the*
425 *International Symposium on Plastics Technology* (pp. 28-36). Springer Berlin Heidelberg.

426 Santos-Beneit, F., Chen, L. M., Bordel, S., Frutos de la Flor, R., García-Depraect, O.,
427 Lebrero, R., ... & Börner, T. (2023). Screening enzymes that can depolymerize commercial
428 biodegradable polymers: heterologous expression of *Fusarium solani* cutinase in *Escherichia*
429 *coli*. *Microorganisms*, 11(2), 328.

430 Shahbazi, M., & Jäger, H. (2020). Current status in the utilization of biobased polymers for
431 3D printing process: a systematic review of the materials, processes, and challenges. *ACS*
432 *Applied Bio Materials*, 4(1), 325-369.

433 Sharma, P., Ahuja, A., Izrayeel, A. M. D., Samyn, P., & Rastogi, V. K. (2022).
434 Physicochemical and thermal characterization of poly (3-hydroxybutyrate-co-4-
435 hydroxybutyrate) films incorporating thyme essential oil for active packaging of white bread.
436 *Food Control*, 133, 108688.

437 Sid, S., Mor, R. S., Kishore, A., & Sharanagat, V. S. (2021). Bio-sourced polymers as
438 alternatives to conventional food packaging materials: A review. *Trends in Food Science &*
439 *Technology*, 115, 87-104.

440 Sim, S. J., Snell, K. D., Hogan, S. A., Stubbe, J., Rha, C., & Sinskey, A. J. (1997). PHA
441 synthase activity controls the molecular weight and polydispersity of polyhydroxybutyrate in
442 vivo. *Nature biotechnology*, 15(1), 63-67.

443 Tanadchangsang, N., & Yu, J. (2012). Microbial synthesis of polyhydroxybutyrate from
444 glycerol: gluconeogenesis, molecular weight and material properties of biopolyester.
445 *Biotechnology and bioengineering*, 109(11), 2808-2818.

446 Tang, T. C., An, B., Huang, Y., Vasikaran, S., Wang, Y., Jiang, X., ... & Zhong, C. (2021).
447 Materials design by synthetic biology. *Nature Reviews Materials*, 6(4), 332-350.

448 Venezia, V., Prieto, C., Evtoski, Z., Marcoaldi, C., Silvestri, B., Vitiello, G., ... & Lagaron, J.
449 M. (2023). Electrospun hybrid TiO₂/humic substance PHBV films for active food packaging
450 applications. *Journal of Industrial and Engineering Chemistry*, 124, 510-522.

451 Wang, J., Zhang, D., & Chu, F. (2021). Wood- Derived Functional Polymeric Materials.
452 *Advanced Materials*, 33(28), 2001135.

453 Zhantlessova, S., Savitskaya, I., Kistaubayeva, A., Ignatova, L., Talipova, A., Pogrebnyak, A.,
454 & Digel, I. (2022). Advanced “green” prebiotic composite of bacterial cellulose/pullulan
455 based on synthetic biology-powered microbial coculture strategy. *Polymers*, 14(15), 3224.

456 Zhao, X. H., Niu, Y. N., Mi, C. H., Gong, H. L., Yang, X. Y., Cheng, J. S. Y., ... & Wei, D. X.
457 (2021). Electrospinning nanofibers of microbial polyhydroxyalkanoates for applications in
458 medical tissue engineering. *Journal of Polymer Science*, 59(18), 1994-2013.

459 Zhou, B., Hu, X., Zhu, J., Wang, Z., Wang, X., & Wang, M. (2016). Release properties of
460 tannic acid from hydrogen bond driven antioxidative cellulose nanofibrous films.
461 *International journal of biological macromolecules*, 91, 68-74.

462 Zieliński, P. S., Gudeti, P. K. R., Rikmanspoel, T., & Włodarczyk-Biegun, M. K. (2023). 3D
463 printing of bio-instructive materials: Toward directing the cell. *Bioactive Materials*, 19, 292-
464 327.