

Current state of the art biotechnological strategies for conversion of watermelon wastes residues to biopolymers production: a review

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Abstract

Poly-3-hydroxyalkanoates (PHA) are biodegradable and compostable polyesters. This review is aimed to provide a unique approach that can help think tanks to frame strategies aiming for clean technology by utilizing cutting edge biotechnological advances to convert fruit and vegetable waste to biopolymer. A PHA manufacturing method based on watermelon waste residue that does not require extensive pretreatment provides a more environmentally friendly and sustainable approach that utilizes an agricultural waste stream. Incorporating fruit processing industry by-products and water, and other resource conservation methods would not only make the manufacturing of microbial bio-plastics like PHA more eco-friendly, but will also help our sector transition to a bioeconomy with circular product streams. The final and most critical element of this review is an in-depth examination of the several hazards inherent in PHA manufacturing.

Keywords: Biopolymers; watermelon; residue; microorganism; bioeconomy.

1. Introduction

Over the years agriculture and horticulture waste production have increase manifold as demands for agriculture products has increased dramatically (Zia et al., 2021). The growing global population has resulted in a multifold increase in agricultural production and a concomitant accentuation in agricultural waste. Available recent report of Food and Agriculture organization stated that 1.3 billion tons of food were wasted during 2020 (Blakene, 2019; Awasthi et al., 2021a). The preference of daily diet in many countries is shifting from food security to nutrition security and this is an important reason that the demand for fruit and vegetable is increasing very fast among humans. Recent reports showed that dietary habits and consumer preferences have led to increasing the production of fruit and vegetables, production figures from 2018 showed that the production of fruit and vegetable exceed by one billion tons. Agriculture waste is defined as unintended waste arising out of agriculture activities that includes crop residues, sawdust, forest waste, orchard waste, leaf litter and weeds. Fruit and vegetable waste are a continuous threat to pre-market to post-market sites. The waste generated from various activities involved in agriculture is a growing concern as it present economic, social and environmental challenges (Reshmy et al., 2021a; Awasthi et al., 2021c).

Watermelon (*Citrullus lanatus*) is an important horticulture crop that belongs to the family of *Cucurbitaceae*, widely grown around the world for its sweet edible fruits and juice (Yadav et al., 2021a, b; Ding et al., 2021). Watermelon is a major yielding crop for the short term and can be grown around the year in protected cultivation. It contributes a large share to the economy in fruit vegetable crops. Botanically watermelon is an annual creeping herb that originated from Southern Africa has attained the level of most economically fruit vegetable crop. Based on the statics of Food and Agriculture Organization the global watermelon production and production area reached approximately 104 million tons and 3084217 ha in

2019, respectively ([Fabani et al., 2021](#); [FAO 2021](#)). In the current situation, watermelon can be grown in almost 122 countries and around 1200 various cultivars are available on all cultivated continents. In terms of production, China produces the highest quantities, followed by turkey, India, Brazil and Algeria. Moreover, China ranks first in production and area of production ([Figure 1](#)). A recent report showed that watermelon is a significant contributor to the agro-based economy of Malaysia ([Kassim et al., 2021](#)). Watermelon production on the commercial is done around the year, and from production to consumption, many kinds of waste is generated. Commercial farms generated a huge amount of waste including leaves, branches, tendrils, unfertilized flowers, tender and diseased fruits during the production and post-harvest waste includes plant biomass. Generally, these wastes are used for animal feed, landfilling, on-site disposal by combustion and direct mulching, which can bring invertible risk from the environment and social point of view. In addition, it has also been reported that such fruit and vegetable wastes promote the microbial risk of disease in animals because fruit waste disposal sites are a habitat for many pathogenic bacteria, fungi, and yeast ([Kartik et al., 2021](#); [Awasthi et al., 2020b](#)).

In current era, safe and sustainable disposal of fruit and vegetable waste have sparked interest in biotechnological strategies aimed at transforming waste material into biomaterial and bioenergy. These strategies can be used to reduce the dependency on fossil fuels for energy demands ([Wainaina et al., 2019](#); [Awasthi et al., 2021b](#)). Transformation of agriculture and horticulture-based waste to energy approach is receiving attention toward the fact that these materials are a rich source of lignocellulosic biomass and available at a lower cost. Moreover, these materials are available throughout the year so, it can be assumed that the supply of such raw material will be continuous ([Bon et al., 2021](#); [Reshmy et al., 2021a](#)). The best way to valorize biomass derived from pre-and post-harvest is the conversion of these materials to biopolymers, such as biogas and bio-hydrogen using microbial processes. As a

matter of fact, variety of bacterial strains are identified that can synthesize biopolymers and convert fruit and vegetable waste materials into valuable extracellular and intracellular bio-products, which are useful for biochemical production ([Amadu et al., 2020](#); [Aaliya et al., 2021](#); [Awasthi et al., 2021c](#)).

Therefore, starting from the available previous considerations, our review offers a state of the current biotechnological strategic approach available for the utilization of watermelon waste residue. This review will provide a unique approach that can help think tanks to frame strategies aiming for clean technology by utilizing cutting edge biotechnological advances to convert fruit and vegetable waste to biopolymer.

2. Nature of watermelon wastes residues

Watermelon fruit consists of water, seed, pulp, peel and rinds. Studies show that around 60-70% of watermelon fruit is edible whereas the remaining 30-40% includes seed, rind and other parts which are not edible and are considered as watermelon fruit waste. Majority of the watermelon produce is consumed as a fresh fruit and remaining is utilized for processing products such as, vinegar, wine and packed juice ([Chen et al., 2017](#); [Liu et al., 2021a](#)). The waste generated from watermelon has very little economic value and is only used for animal feed and landfills in many countries. Consequently, huge amount of watermelon waste is discarded, mostly in summer the waste amount is comparatively higher than other seasons. A report showed that solid waste volume is higher as much 39,000-52,000 tons/year in Argentina alone ([Fabani et al., 2021](#)). However, the seeds are edibles and may constitute part of nutraceutical when consumed as a food supplement. It is an important source of protein, dietary fibers, minerals, antioxidants, and other micronutrients ([Wainaina et al., 2019](#); [Cal et al., 2021](#)). It has been already identified that watermelon rind waste contains carbohydrates and fibers in the large amount. If we consider the waste generated from watermelon from farms and greenhouses, the leaves, stems, branches and tendrils are

also important waste generated along with fruits. To date, revolutionary progress has been attained in agro-waste processing and many approaches introduced to waste to energy perspective (Liu et al., 2021b; Reshmy et al., 2021b). The watermelon waste can be converted into valuable products including bioremediation, bio-sorbent, biochar, and dietary food. Watermelon waste mainly rind is discarded in large amounts is consists of lignocellulosic polysaccharides including cellulose, pectin, hemicellulose and lignin, low molecular and carotenoids material such as citrulline and other phytochemical compounds.

2.1. Pre-and post-harvest watermelon waste

The watermelon cultivation is a long process and required various cultivation practices. Overall, the waste can be categorized in two types i.e., pre- and post-harvest waste (Figure 2). Pre-harvest waste are recovered in growing duration of watermelon in field as well as greenhouse cultivation. Pre harvest waste is basically leaves, dropped flowers, stem, tendrils and pruned branches. The biomass generated during cultivation is generally underutilized products. Close observation of cultivation practices shows that their wastes are zero value products because not collected for commercial bio-product conversion and disposed in farm or used as animal feed. Post-harvest waste includes watermelon rotten fruits, rind/peel and unutilized seeds. A study revealed that approximately 36 million tons of watermelon rind was generated during 2013 (Petkowicz et al., 2017; Liu et al., 2021b). In the last decades, many literatures showed that these wastes have huge potential for industrial utilization. This waste can be used to recover many biochemical compounds.

2.2. Composition of watermelon waste residue

Watermelon waste is categorized into three major parts, which are rind, seed and flesh. The rind and seed contributed to the majority of waste. According to the production data of 2017-18, close to 42 million tons of watermelon seed and rind waste were generated from various processing industries alone (Zia et al., 2021). A thick white flesh between the inner

peel and outer green cover is known as watermelon rind. It contributes to almost one-third of the total fruit weight and is generally discarded due to its unappealing flavour. Watermelon peel waste consists of many major carbonaceous components, including cellulose and hemicellulose, indicating that it can be utilized as a raw material for lignocellulosic biomass to fuel production. In general, watermelon rind waste is not used and it is considered as a zero-value waste. However, some reports showed that rind waste products are being used for various purposes like stewing, stir-frying and often picked in many growing areas (Liu et al., 2021b). Furthermore, dry powder produced from watermelon rind showed innovative alternative to nontraditional flours (Hoque and Iqbal, 2015; Wainaina et al., 2020a). Some previous reports showed that watermelon rind contains 32.2% carbohydrate, 26.3% crude fibers, 5.0% ash, 12.4 % crude protein and 12.6 % lipid (Awasthi, et al., 2021a). The proximate composition of seed and peel are presented in Table 1 based on the previous reports. Watermelon peel waste has been used to produce lipid production due to high C/N ratio 21.59%, which makes it ideal target for lipid production (Hashem et al., 2020; Sindhu et al., 2021). It was estimated that it can also be used for production of many other value products. Till date, not much information is available on biofuel production from watermelon waste. In previous study of Cha et al. (2020) reported that 4.16 gL⁻¹ (approx.) bioethanol was recovered after co-saccharification and fermentation of watermelon rind waste by *Trichoderma viride*. A recent study by Ratnakaram et al. (2020) confirmed that bioethanol can be produced from fermentation by using two different species of *S. cerevisiae*, and higher production yield can be achieved by toddy origin yeast strains.

3. Pretreatment methods and Recovery of biopolymers from watermelon wastes residues

Most of the part of the watermelon is edible, while the rind has no commercial value, and it is considered a waste. Watermelon waste residue consists of cellulose and

hemicellulose, the rich source of carbon feedstocks for microorganisms. Utilizing these components can play a significant role in synthesizing functional and biodegradable biopolymers (Kassim et al., 2021). Watermelon wastes can be obtained from various juice production agro-based industries. For processing, the watermelon wastes should be washed in distilled water and cut into small pieces. The obtained pieces should be dried and ground into a fine powder. The dried substrate cannot be used directly for biopolymer production. Therefore, it needs a pretreatment step. The pretreatment of watermelon waste residues is done by soaking it in 0.7 % (v/v) sulphuric acid and autoclave for 60 min (Kassim et al., 2021; Awasthi, et al., 2021a; Luo et al., 2021). After pretreatment, the mixture is centrifuged, and the solid residue is washed with hot water and dried in an oven for 24 hours at 60° C. Cellulose and hemicellulose content of the material is degraded by the respective enzymes cellulases and hemicelluloses (Wainaina et al., 2020a; Sindhu et al., 2021). The overall biopolymer production process from the watermelon waste residue is presented in Figure 3. In the process, the watermelon waste or rind is first pretreated using appropriate treatment procedures. After pretreatment, a low-cost culture medium is designed and used with a high biopolymer accumulating microbe to produce biopolymer. After production, the cells from the culture media are harvested, dried, and the polymer is extracted in reflux conditions. Post extraction, the biopolymer is precipitated and dried. The final product obtained is a biopolymer. Later, the biopolymer can be modified as per the requirement of the specific application.

3.1. Extraction of biopolymer from microbes

After completion of culture growth, the microbial cells are harvested and freeze-dried. The freeze-dried cells are subjected to an extraction procedure. There are several methods available for the isolation of biopolymers from cells. These include solvent extraction,

microwave extraction, ultrasound extraction and subcritical water excretion. Each of the methods is discussed below.

3.1.1 Solvent extraction

This method can be used for the extraction of biopolymers from bacterial and algal biomass. This process is easy and needs a few downstream processing steps. In algae, the biomass can be combined to form polymer precipitates (Roja et al., 2019). Chemical and physical parameters optimization enhances the polymer accumulation. The polymer alginate was extracted using mineral acids (pH 1.5) from the algae *Padina pavonica* (Faidi et al., 2019). The extraction process relies on operations such as filtration, sifting, and centrifugation. A study screened six microalgae stains, and it was observed that three of the strains *Synechocystis* sp., *Nostoc* sp. and *Porphyridium purpureum* had higher biopolymer synthesis (Morales-Jiménez et al., 2020).

3.1.2 Microwave mediated extraction

This method is a unique and green approach for the recovery of biopolymers microalgae. Microwave assisted extraction has various advantages, such as it is quick, compact, with short experimental times and require no energy. A hybrid carrageenan biopolymer was extracted from red algae *Mastocarpus stellatus* using a microwave (Ponthier et al., 2020; Awasthi et al., 2021b). The process resulted in a high yield of biopolymer. The maximum yield of biopolymer was obtained at 150 °C for 6 min. Increased stability in terms of strength was observed with increased temperature. This method suggests a very short time range for biopolymer extraction. Therefore, it can be used at a large scale for biopolymer extraction.

3.1.3 Ultrasound mediated extraction

Ultrasound mediation biopolymer extraction is based on the phenomenon of cavitation. The phenomenon of cavitation causes a collision in the microbial cells of

microalgae. Collision and agitation in the microalgae cells lead to cells disruption and thereby release of the content. In the process, the transfer rate is increased, which results in the removal of biopolymers (Jain et al., 2022). Ultrasound-assisted extraction has several advantages such as reduction in time of extraction, operation at room temperature, reduces loss of materials, is ecofriendly and no requirement of membrane separation. As compared to the traditional methods, the ultrasound-assisted method gives a higher extraction yield. Ultrasound assisted extraction method was used for isolation of the biopolymer alginate from *Sargassum muticum* (Flórez-Fernández et al., 2019; Sindhu et al., 2021). It was observed that the ultrasound method of extraction was four times efficient as compared to the conventional methods because it reduced the extraction time by four times. The extraction efficiency was affected by parameters such as temperature, sonication time and ultrasound frequency (Wainaina et al., 2020b; Awasthi, et al., 2021b). It was observed that with a higher sonication process, the biopolymer yield was increased. Increase in sonication frequency and sonication times lead to increased biopolymer yield.

3.1.4 Subcritical water extraction

It is an emerging technique for the isolation of bioactive compounds from different biomass. The method utilizes water that is heated to more significant than the boiling temperature and pressurized below critical pressure (Gereniu et al., 2018; Qin et al., 2021a). There are several techniques available for biopolymer extraction, but subcritical water extraction has some unique advantages. It uses water as a solvent, which surpasses the use of disruptive chemicals for extraction. Other advantages include short retention times, high yield, high quality, and low energy consumption (Alboofetileh et al., 2019; Guo et al., 2021). A study involved extraction of biopolymer fucoidan from *Saccharina japonica* with subcritical water extraction (Saravana et al., 2018; Jayakumar et al., 2021). The study demonstrated that subcritical water extraction was a much efficient procedure as compared to the conventional

extraction procedures. In another study, fucoidan was isolated from *Nizamuddinina zanardinii* using subcritical water extraction (Alboofetileh et al., 2019; Liu et al., 2021). The results demonstrated that subcritical water extraction was much better technique as compared to the traditional methods.

4. Biotechnological strategies for biopolymer production

Watermelon waste residues contain various carbon and nitrogen sources. This carbon and nitrogen (C/N) ratio play an important role in the microbial production of biopolymers utilizing microelements. The residues obtained from the watermelon waste can be used to design a low-cost microbial culture medium. Later, this culture medium can be optimized to maximize the production yield of the biopolymer. The cost of using a synthetic medium for biopolymer production is very high. Therefore, low-cost culture media from waste will be a panacea in the biotechnology of polymer production. PHAs are most popular. It is estimated that the PHA market will achieve US \$ 93.5 million in 2021 from US \$ 73.6 million in 2016 (Singh et al., 2019). Moreover, an additional expectation of reaching US \$ 18.66 million from 2020-2024 (Gahlawat et al., 2020; Guo et al., 2021). For this reason, most of the research is focused on the strategies to maximize their production utilizing economic substrates in recent years. These substrates are mainly wastes originated from agricultural industries. Discussed below are the major strategies and parameters which need to be considered while maximizing the biopolymer production in microbes. Figure 4 presents the biotechnological strategies involved in biopolymer production.

4.1. Biopolymer production routes

In bacteria, PHA synthesis takes place via various metabolic rates. For example, heterotrophic bacteria utilize extracellular substrates. The photoautotrophs utilize CO₂ as a substrate to synthesize metabolites. These microbes produce the products in anaerobic conditions, which can be a cost-effective approach to eliminate oxygen requirements in the

culture conditions (Fradinho et al., 2019; Qin et al., 2021a). Biopolymer is produced in nitrogen starvation conditions. In stress, the amino acid synthesis is reduced. Thereby a concomitant increase in the acetyl-CoA flux and PHA synthase. In the PHA heterotrophic production route, microbes such as *Spirulina* sp., *Halophilic archaea* and *Synechocystis* sp. used organic matter from waste. In the chemotrophic PHA production route, microbes utilize chemicals such as acetate for PHA precursors. Therefore, this route is expensive as compared to others. In contrast, phototrophic bacteria and microalgae sequester the carbon from CO₂, which makes the procedure cost-effective for PHA production (Carpine et al., 2020; Qin et al., 2021b). In some instances, such as in *Cupriavidus necator*, it was observed that poly hydroxybutyrate (PHB) could be produced both heterotrophically and heterotrophically (Xu et al., 2021; Xu et al., 2020). Utilization of organic substances took place in *Cupriavidus necator* heterotrophically. It was observed that during the nutrient limitation conditions, PHB production was performed utilizing organic carbon.

A study on PHB production using *Rhodopseudomonas palustris* TIE-1 demonstrated that PHB yield was higher in aerobic chemoheterotrophic conditions (Ranaivoarisoa et al., 2019). Photoheterotrophic growth of *Chlorella Vulgaris* had better growth as compared to the shake flask conditions. In contrast, *Spirulina* sp. LEB 18, *Nostoc muscorum* and *Calothrix cytonemicola* had less efficient growth performance in photo-bioreactors (Carpine et al., 2020). Although, various studies were conducted using pure single strains of microbes for PHA production (Oliveira-Filho et al., 2021). But mixed microbial culture is also advocated due to advantages such as low-cost operation and complex substrate utilization (Fradinho et al., 2019; Perez-Zabaleta et al., 2021). Apart from the use of indigenous microbes for PHA production, approaches such as metabolic engineering for alteration of biosynthetic pathways have been practiced (Cal et al., 2021; Liu et al., 2021; Zheng et al., 2020). The strategy involves overexpression of PHA synthesis genes, thereby enabling the microbe to metabolize

utilization of a wide range of substrate range. In this way, microbes are adapted to utilize a variety of wastes originated from varying sources.

4.2. Selection of biopolymer producing microbes

PHA accumulating microbes have been adapted to survive in extreme environments, including wastewater, soil, hydrothermal vents, and salt springs (Kumar et al., 2020; Tan et al., 2021; Yadav et al., 2021b; Zheng et al., 2020). These characteristics for their survival in extreme environments make them extremophile. Halophiles can survive in a very high salt concentration. The advantage of using halophiles is that no sterilization culture medium is required (Liu et al., 2021a; Obulisamy and Mehariya, 2021). Both prokaryotic, as well as eukaryotic microbes, are known to thrive well in organic matter-rich environments (Amadu et al., 2020). It has been observed that PHB producing microbes cell integrity is also protected by the biopolymer (Sedlacek et al., 2019; Awasthi, et al., 2020; Qin et al., 2021b, c). This happens because the presence of hydrophobic molecules in the bacterial cell membranes provides with added flexibility and ionic balance to the membrane. It will be an advantageous approach to screen the microbes from extreme habitats such as high salt concentration, high temperature or osmotic pressure. This is because extreme environments exert additional stress on cell physiology. Thereby inducing enhanced biopolymer accumulation and storage. There are various quick and easy methods available for screening PHA accumulating microbes (Tripathi et al., 2021; Shahid et al., 2021). Moreover, microbes can also be classified based on the conditions, such as whether they require essential nutrients like nitrogen or phosphorus. A few bacteria such as *Pseudomonas oleovorans*, *Protomonas extorquens* and *Cupriavidus necator* requires essential nutrients and their deficiency leads to PHA accumulation. Some other bacteria, such as *Alcaligenes latus* and recombinant *Escherichia coli* are unaffected by presence or absence of essential nutrients (Amadu et al., 2020).

4.3. Genetic manipulation

The microbial strains can be engineered to modify the substrate range for PHAs production. The strains are also modified in cell morphology for easier downstream processing. PHB synthesis in bacteria takes place via the key enzymes such as PHB synthase, NADP-specific acetoacetyl-CoA reductase, β -ketothiolase which codes for PHB genes. PHB copolymers was produced by modifying the fatty acid amounts in the engineered *Pseudomonas putida* KTQQ20 stain (Amadu et al., 2020; Awasthi et al., 2020a). The *E. coli* cells can be modified in such a way that it will switch to multiple fission as compared to binary fission. In this way, bacteria is able to accumulate higher proportions of PHB in the cells (Wu et al., 2016; Duan et al., 2020; Guo et al., 2021). An increment in dry cell weight was also observed. Gene manipulation can also be made to transfer PHB genes from a native host such as *Ralstonia eutropha* to a non-native host such as *Chlamydomonas reinhardtii*. Also, the complete PHB pathway of *Ralstonia eutropha* was successfully integrated into a diatom to achieve a higher PHB (Amadu et al., 2020). A review discussed the engineering PHA biosynthesis for cost reduction and diversity (Zheng et al., 2020). In a study, *Pseudomonas putida* metabolic engineering was performed to produce several short-chain lengths PHAs types from levulinic acid (Cha et al., 2020; Qin et al., 2021c). Another study demonstrated *Pseudomonas mendocina* NK-01 metabolic engineering for improved MCL-PHAs (Zhao et al., 2020). A study was focused on the analysis of whole-genome sequencing of *Cupriavidus* sp. ISTL7 for PHA production (Duan et al., 2020; Gupta et al., 2021).

4.4. Optimization of microbial growth parameters

Biopolymer accumulation and production depends on various parameters such as strain type, nutrient composition, feeding mechanism, pH of the medium, photoperiod and cycle length. The carbon feedstock or substrate plays a major role in biopolymer production. But type and mode of substrate application have a critical role in driving the microbial

community performance. In a study, cyanobacteria (*Leptolyngbya*, *Synechococcus*, and *Oscillatoria*) and microalgae (*Chlorella*) were grown in ASN III medium (Roja et al., 2019; Qu et al., 2021). The study demonstrated that cyanobacteria grow faster in the medium as compared to microalgae. PHB accumulation in microbes is dependent on the limitation of nitrogen and phosphorus. The highest PHB accumulation has been experienced with an overall combined effect of nitrogen and phosphorus deficiency. The cyanobacteria have a higher growth rate as compared to green algae *Scenedesmus* sp. in sequential batch reactor (Arias et al., 2019; Duan et al., 2021). In the absence of phosphorus or nitrogen, the bacteria do not produce proteins. But produce and accumulate PHA in the form of enclosed granules with excess of carbon.

5. Biopolymers and their composites

Biopolymers can be classified as biopolymer monomers derived from microbes, biopolymer blends, biopolymer composites, biopolymer hybrid systems, and PHAs and their blends. Each of the categories has been discussed below. Table 2 presents various types of biopolymers and their composites.

5.1. Biopolymers derived from microorganisms

These are biopolymers which originated from microorganisms and microalgae. They are alginate, bacterial cellulose, β -glucans, xanthan and PHAs. They have been described below, along with their sources.

5.1.1. Alginate

Alginates are the biopolymers produced by algae. Alginates find their applications in the areas such as biomedicine and food (Devadas et al., 2021; Kartik et al., 2021). The physiological properties of alginates vary depending on their source of origin. In general, they are linear chain consisting of α -L-guluronic acid and β -D-mannuronic acid joined with each other with 1-4 linkages. Alginates are biocompatible and environment safe (Ahmad et

al., 2021; Wang et al., 2019). Generally, alginates can be isolated from marine seaweed and brown algae. It has been observed that it can also be produced in a few bacterial species genera such as *Azotobacter* and *Pseudomonas*. There are various areas where alginates find their applications. These include thickening, gelling and stabilizing agents (Martău et al., 2019). They are also used to produce hydrogel beads, high value products, and biomedical devices (Ahmad et al., 2021; Chandra et al., 2019; Murujew et al., 2021).

5.1.2. Bacterial cellulose

In general, bacterial cellulose is produced by gram negative bacteria genera such as *Acetobacter*, *Azotobacter*, *Archomobacter*, *Alcaligenes*, *Agrobacterium*, *Rhizobium*, *Pseudomonas*, and *Salmonella*. But it is also produced by a few gram positive bacteria such as *Sarcina* (Horue et al., 2021). The plants can also produce cellulose. But bacterial cellulose has some unique properties such as high polymerization degree attributed by glucan, greater percentage of crystallinity and high-water retaining capacity. The above properties provide it with a higher thermal stability and mechanical properties. Bacteria from the genus *Komagataeibacter* are known to produce high quality cellulose with excellent efficiency. The crucial element in their cellulose productivity is the synthetic medium known as Hestrin-Schramm medium. The cost of cellulose production is not affected in a laboratory scale. But when it comes to a commercial level, the production becomes highly expensive. This limits the commercialization of cellulose production. Therefore, alternative carbon feedstock's such as agro-wastes including watermelon wastes are considered as promising candidates for the cellulose production (Gayathri and Srinikethan, 2019; Hussain et al., 2019; Kumar et al., 2019; Wang et al., 2019). Therefore, development of a suitable production medium along with suitable microbial selection is a mandatory requirement.

5.1.3. β -glucans

β -glucans consists of glucose monomers which are connected by β glycosidic bonds at 1-3, 1-4 or 1-6 linkages. Types of bonding determine the property of the polymer and hence its applications. β -glucans exhibit various useful properties such as excellent water-absorbing capacity, antioxidant capacity, anticancer properties, immune stimulation and lipid-lowering effects (Yuan et al., 2020). This polymer is produced by microorganisms such as bacteria, fungi, and yeast (Kaur et al., 2020). It can be considered as an important candidate for medical applications such as wound healing, cancer therapy and drug delivery systems (Molina et al., 2019; Vetvicka et al., 2019; Yuting et al., 2020).

5.1.4. Xanthan

It is a hetero-polysaccharide consist of glucose monomers linked with β -(1-4) glycosidic bonds. It has additional branching with mannose and glucuronic acid. Moreover, glycosylation gives it an anionic property (Patel et al., 2020). Xanthan have various useful properties, such as it is soluble in cold and hot water, has great ionic strength, and it is stable at low pH (Elella et al., 2020; Ravindran et al., 2021). Xanthan is produced as exopolysaccharide by the bacterium *Xanthomonas campestris*. The bacterium can produce the biopolymer by using glucose as carbon source. But the glucose is very expensive as a production medium. Therefore, alternative carbon feedstock such as watermelon waste can serve the purpose.

5.1.5. Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates are the most popular biopolymer, and they compete the synthetic polymer in terms of properties and reliability. This polymer can be synthesized in laboratory conditions utilizing superior bacterial species such as *Pseudomonas*, *Bacillus*, *Streptomyces*, *Alcaligenes*, and *Azotobacter*. It can also be produced by the genetic manipulation of *E. coli* (Magagula et al., 2021). Bacteria produce PHAs in response to

nitrogen and phosphorus stress. PHAs accumulate in their stationary phase of growth (Sarsaiya et al., 2018; Müller-Santos et al., 2021). PHAs can be produced from various feedstocks such as wheat bran, cheese whey, glycerol waste, fruit wastewaters and waste cooking oils (De Donno Novelli et al., 2021; Kalia et al., 2021; Magagula et al., 2021; Sirohi et al., 2021). PHAs finds applications in various industries ranging from food packaging to drug delivery (Tan et al., 2021).

5.2. Biopolymer blends and composites

Biopolymer characteristics can be enhanced by combining them with some useful and efficient materials. Combinations make them more efficient and reliable for various applications. They are known as hybrid systems or composites. Biopolymer composite, a heterogeneous mixture of various materials where the individual components contribute to provide the material with superior properties such as durability, biocompatibility, degradability, adaptability and mechanical strength (Sarsaiya et al., 2019; Aaliya et al., 2021). Biopolymer composites can be prepared by interactions such as electrostatic interactions, covalent bonding, and hydrogen bonding etc. It has been observed that the biopolymer composites have voids in between the molecules. The presence of these voids is a beneficial characteristics because the voids helps in faster recovery of damaged tissue in scaffold in tissue engineering (Horue et al., 2021; Singhania et al., 2021).

Alginate composites can be prepared by the addition of materials such as collagen, divalent cations such as Ca^{2+} and gelatin. The addition of collagen makes it suitable for application such as prosthesis coating (Horue et al., 2021). While the addition of divalent cation makes it perfect for encapsulation (Ramdhan et al., 2020; Wang et al., 2021). Addition of gelatin makes it a suitable material for use as hydrogel for tissue engineering (Horue et al., 2021). Bacterial cellulose can be combined with other materials such as alginate and chitosan to perform applications such as drug delivery (Cabañas-Romero et al., 2020). Carrageenan

can be combined with lignin and other materials for application in drug delivery and food packaging (Numata et al., 2021; Rukmanikrishnan et al., 2020). Casein can be combined with alginate and chitosan to form nano-fibers and scaffold for applications in tissue engineering and drug delivery (Du et al., 2020; Mishra et al., 2021). Sodium carboxy-methyl xanthan gum can be combined with N-trimethyl chitosan to form hydrogel for drug delivery applications (Hanna and Saad, 2019). Collagen biopolymer can be combined with alginate to use in the drug delivery systems in the gel applications (Mei et al., 2019; Xu et al., 2021).

The biopolymer poly-3-hydroxybutyrate can be combined with humic acid and siRNAs in the form of nanoparticles for applications in detoxification system and gene delivery system, respectively (Hamza et al., 2019; Lee et al., 2020). Biopolymer composites can also be prepared by combining polymer with inorganic compounds. For example, bacterial cellulose can be combined with montmorillonite and iron oxides to form biopolymer-inorganic hybrids for applications in wound dressing and food processing, respectively (Horue et al., 2020; Jia et al., 2021). β -glucan can be combined with silver and hydroxyapatite to form a porous scaffold for use in tissue engineering (Khan et al., 2020; Duan et al., 2021). Poly-3-hydroxybutyrate can be combined with calcium carbonate, alginate, and hydroxyapatite to form a fibrous scaffold for applications in tissue engineering and drug delivery (Chernozem et al., 2019; Volkov et al., 2020).

5.2.1 Biopolymer hybrid systems

There are various types of hybrid systems available depending on the type of biopolymer used. Alginate being a versatile biopolymer can form hybrids in different structures and shapes such as coatings, hydrogels, micro-particles and films. The alginate-collagen hybrid system can be prepared to cover the prosthesis. It was observed that the produced hybrid system has the properties like that of the extracellular matrix, which have a high affinity to the osteoblast cells (Horue et al., 2021). The hybrid system can be used in the

cancer treatment ([Mei et al., 2019](#)). 3D bio-printing have been used to produce a matrix of alginate and gelatin to study tumor progression status in breast and fibroblast cells. A recent study prepared a sol-gel composite with silica and alginate for applications in tissue engineering ([Vueva et al., 2018](#); [Xu et al., 2020](#)).

Bacterial cellulose has excellent biocompatibility and versatility. This is the reason; it can combine with different materials to form composites. There are two approaches that are used to modify the bacterial cellulose. In the first approach, the membranes which have been already modified are placed in a modifier solution compound. The modifier solution may contain compounds such as nanoparticles, metal oxides and other polymers. The reaction results in the formation of a novel hybrid material. In the second approach, the modifier solution is added directly to the culture medium where they interact with the growing polymeric chain modifying the self-assembly ([Gorgieva and Trček, 2019](#)). Inorganic materials such as silver, titanium oxide, silica or montmorillonite clay may also be used to modify the bacterial cellulose membranes to produce the hybrid inorganic composites. This type of modification results in the formation of a composite with superior chemical and mechanical properties with antimicrobial properties ([Gorgieva and Trček, 2019](#)). Silver cations from the montmorillonite can be used for modification of bacterial cellulose ([Gorgieva, 2020](#)). Bacterial cellulose and chitosan were utilized to produced composite papers which show antimicrobial activity, antioxidant properties and durability for use in the applications such as food packaging and biomedical applications ([Cabañas-Romero et al., 2020](#); [Liu et al., 2021a](#)).

Oxidation of bacterial cellulose has a greater effect on the chitosan electrostatic coupling. This helps in a faster and efficient self-assembly. The produced composite show excellent pro-coagulant activity, stability on internal bleeding and enhanced tissue healing in rat models ([Yuan et al., 2019](#)). Bacterial cellulose does not possess a good drug holding

properties for small therapeutic proteins or molecules. That is why it cannot be directly used for drug delivery applications. Instead, it can be modified with some carrier such as methoxylated pectin which have higher drug retention properties. Such composite have a very higher retention potential for molecules such as human serum albumin when compared to the bacterial cellulose alone (Cacicedo et al., 2018; Guo et al., 2021). The cellulose nano-fibers when combined with alginates chains produce a composite with a great response to voltage and pH (Fernandes et al., 2020).

K-carrageenan when combined with materials such as lignin, exhibit some important characteristics such as enhanced mechanical resistance, antioxidant activity and biocompatibility in various mammalian cell lines. The hybrid material finds applications in biomedical devices and packaging applications (Rukmanikrishnan et al., 2020). K-carrageenan when combined with ϵ -polylysine results in an improved magnetic material with strong antimicrobial properties against the microbes responsible for food spoilage (Jia et al., 2021). The drug loading capacity of K-carrageenan can be increased by forming a CaCO_3 microsphere. The formation of microsphere leads to high doxorubicin loading potential. K-carrageenan when added to bacterial cellulose membrane, it forms a biphasic system which have a high tensile strength and slow drug-releasing properties (Numata et al., 2021).

Casein can be used in the forms such as nano-emulsions, nano-micelles, hydrogels, and films. It has self-healing properties. Therefore, it has been explored as a drug delivery system. But one major drawback with the use of casein is that it ruptures with the release of it encapsulated material due to actions of the proteases present in the nature (Rehan et al., 2019; Liu et al., 2021a). Various strategies have been adapted to overcome this problem. Cross linking can be applied to achieve the target. But crosslinking is an expensive approach, and its purification adds more to the cost. Preparation of hybrid system is one of the most suitable approaches where casein and chitosan are used to form an amphiphilic nanoparticle. The

produced nanoparticles have excellent properties such as stability, longer drug retention capacity and potential to encapsulate both hydrophilic as well as hydrophobic substances (Du et al., 2020). A combination of Chit amine group and casein phosphate group was used to form a hybrid fiber. The produced material exhibited good properties such as hemostatic and enhanced mechanical properties (Mishra et al., 2021).

Chitosan finds its application in tissue engineering because of its inherent mechanical properties antimicrobial and anti-inflammatory properties (Cavallaro et al., 2021). In hybrids made of clay and chitosan, chitosan assembles around the clay nano-plates and creates a channel for active compounds for bone tissue regeneration promoting cell proliferation, cell adhesion and cell differentiation (Horue et al., 2021). Chitosan along with hydroxyapatite can be used for re-mineralization of tooth enamel. Hybrids made of chitosan and bacterial cellulose were used to produce a material with reversible shape memory property (Zhu et al., 2019). The diameter of the produced fiber can be regulated by controlling the ionic force and the polymer ratio.

Pectin amphiphilic nature makes it a versatile material for reaction with various other molecules and materials. Pectin methoxylation also impacts its self-assembly properties (Wang et al., 2020). Pectin can form different structures such as micelles, dispersions and polymer networks and shapes such as sphere, mushroom shape, doughnut shape and ellipse. Pectin can be combined with chitosan to improve the tensile strength and thermal resistance. The production of such hydrogels does not require a cross-linker. Rather, they are stabilized by electrostatic coupling and intermolecular association (Martins et al., 2018; Liu et al., 2021a). Xanthan can be combined with various other materials such as metal oxides, nanoparticles, liposomes, polymers and proteins to form composites (Elella et al., 2020; Patel et al., 2020). Composite prepared from xanthan and chitosan can be used to carry ciprofloxacin (Hanna and Saad, 2019). The composite did not show any sign of cytotoxicity

to the normal human lung cell line. In a study, xanthan was combined with ZnO micro-particles. The produced hybrid system had antibacterial activity, was biodegradable, non-toxic and safe (Joshya et al., 2020). PHAs hybrid systems preparation approach requires the combination of a compound with diverse physicochemical properties. This results in the development of a hybrid system with multifunctional properties. This multifunctional approach finds applications in the generation of tissue engineering scaffolds. For example, a scaffold prepared from Poly-3-hydroxybutyrate, poly(ϵ -caprolactone) and 58S sol-gel bioactive glass. The prepared scaffold supported the osteoclast cell proliferation, bone healing, cell adhesion (Chernozem et al., 2021). A scaffold prepared from poly-3-hydroxybutyrate, hyaluronic acid, alginate hydrogel and mesenchymal stem cells demonstrated a very high bone regeneration capacity (Volkov et al., 2020).

5.2.2. PHAs blends and composites

PHAs are highly biocompatible, biodegradable and can be produced from various renewable resources (Chen et al., 2020; De Donno Novelli et al., 2021; Tan et al., 2021). They are produced in nutrient limited conditions such as in deficiency of nitrogen, phosphorus, or magnesium with an excess of carbon. The culture medium is optimized in such a way that the C/N ratio is maintained. PHAs finds application in various areas including packaging, agriculture, tissue engineering, drug delivery and medical bio-plants (Giubilini et al., 2020; Vahabi et al., 2019; Reshmy et al., 2021a, c). There are different types of PHAs available. These include poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV), poly(3-hydroxybutyrate) (P3HB), poly-4-hydroxybutyrate (P4HB) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (6HHx). In recent years, PHAs nano-composites have been used in additive manufacturing or 3D printing. It is a reliable technique to manufacture customized products with complex shapes to be used in multipurpose devices.

Various PHAs composites used in additive manufacturing with their potential applications are listed in [Table 3](#).

6. Research needs and future directions

The production of high-value products from fruit residue wastes has captured the interest of researchers from private as well as public sector in recent years, as a paradigm shift in production is necessary to account for social and environmental consequences. Technological advancements have significantly aided in the advancement of these concepts by optimizing molecular physicochemical characterization and purification procedures. In this respect, biopolymers may either be extracted directly from agricultural wastes or synthesized by microorganisms utilizing waste products as the primary growth medium. The yield of biopolymers is dependent on which of these leftover products is employed, and more research is needed to produce a better appropriate medium for effective large-scale manufacturing with the added benefit of recycling watermelon waste ([Horue et al., 2021](#)). The major research needs are as follows:

- Additional research is required on the migration of elements from various organic-based products under the most prevalent process settings ([Andler et al., 2021](#)).
- Extensive research has been conducted to optimize the energy and time required for PHA extraction and purification in light of associated environmental issues ([Awasthi et al., 2021](#)).
- The dried substrate from watermelon waste cannot be utilized directly for biopolymer synthesis. As a result, more study on the pretreatment phase is required ([Kassim et al., 2021](#)).

In future directions, two major elements of biopolymer synthesis from waste materials have been discussed: purity and hybrid systems. This partial conclusion demonstrates how critical it is to integrate fundamental and applied science in order to optimize the production

of well-established new nano-hybrid biomaterials ([Horue et al., 2021](#)). While LCA studies suggest that PHA manufacturing consumes less energy than synthetic polymers, more environmental indicators may be required to assess the technical progress of PHA processes utilizing fruit wastes. By tying these sectors together and making fruit wastes available as a feedstock, resource utilization may be maximized and hazardous pretreatments associated with other PHA sources can be avoided. In this manner, both production costs and environmental effects may be decreased, resulting in a cleaner manufacturing process while also closing the loop on a waste stream, therefore transitioning to a circular bio-economy ([Andler et al., 2021](#)). Concentrated research of important marketed features might increase the efficacy of biopolymers manufacturing in a cost-effective process:

- The imperative is to develop green and sustainable technology capable of resolving this escalating and pervasive challenge.
- Rapid energy consumption growth and environmental degradation have compelled to retreat and transition from a linear economy (reliant on fossil fuels) to a circular bio-economy.
- By merging scientific study, engineering, and management, it is necessary to use watermelon waste leftovers in order to generate bio-products ([Lee et al., 2020](#)).
- Pectin produced from watermelon peel has the potential to be developed into novel biofilm materials, and that ultrasound may be used to enhance the performance of pectin films and therefore expand their food packaging applications ([Guo et al., 2021](#)).

7. Conclusion

In conclusion, watermelon waste residues have been identified as an organic waste resource that does not require costly pretreatment and may be utilized directly or in combination with microorganisms during the fermentation process. This research provides an

overview of watermelon waste leftovers and their potential as an alternative biopolymer, bioenergy source, and bio-product for more environmentally friendly bio-economic manufacturing processes. The development of hybrid biopolymer systems and composites with specified and customized functions will result in the creation of new materials for tailor made applications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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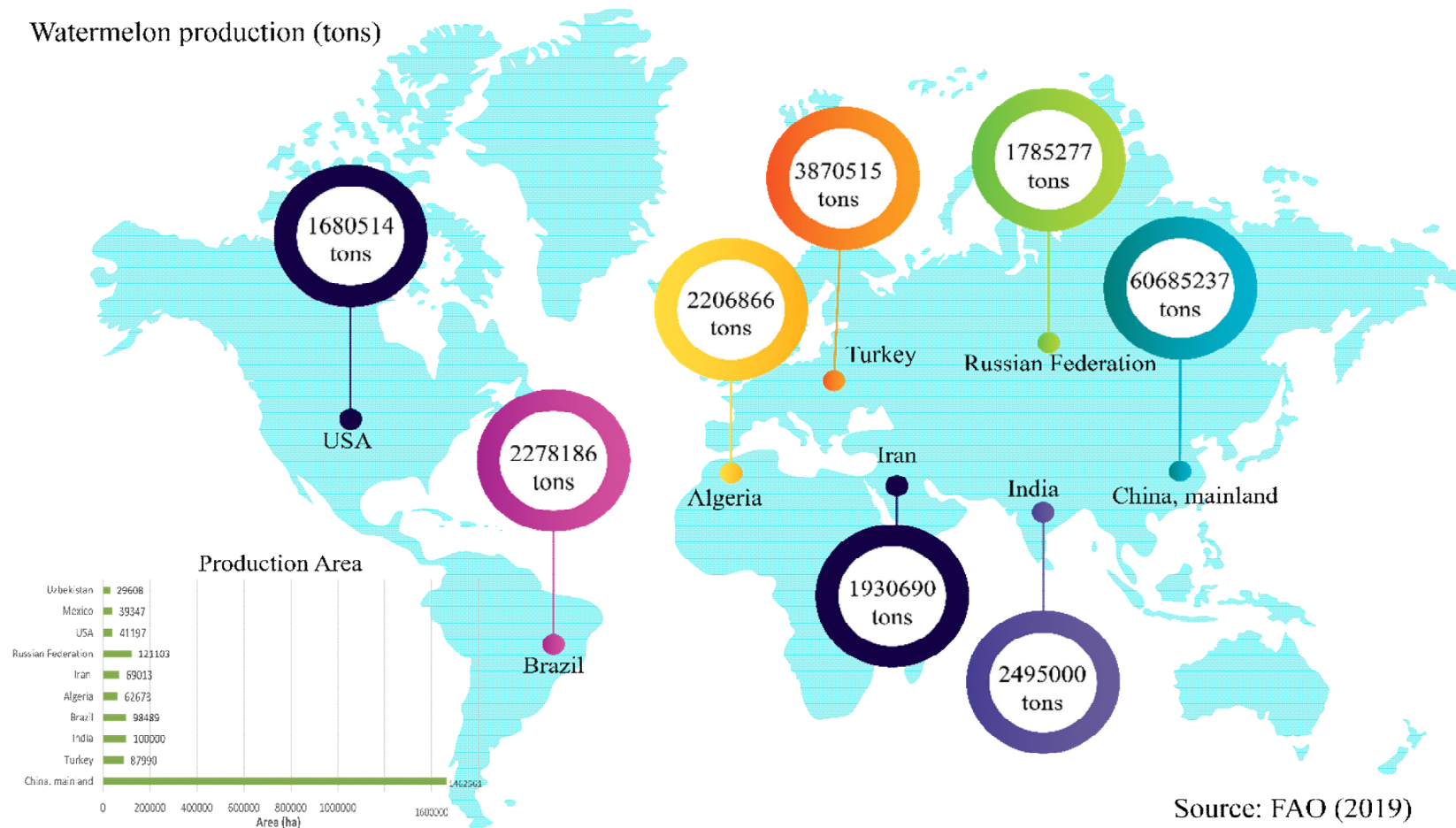
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Watermelon production (tons)



Source: FAO (2019)

Fig. 1. Leading countries in area and production of watermelon (<http://www.fao.org/faostat/en/#data/QCL>, accessed on August 15, 2021)

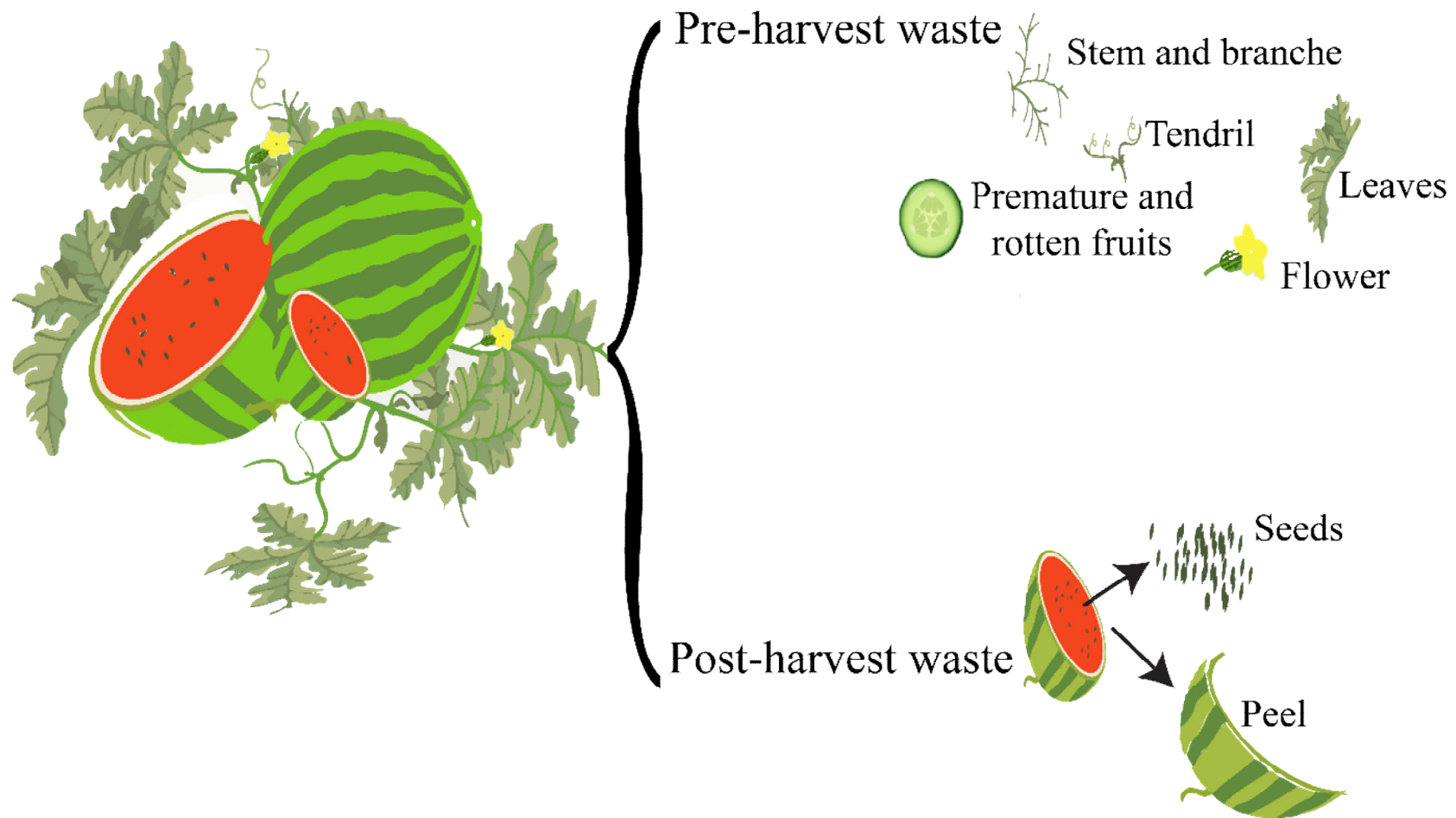


Fig. 2. Watermelon waste generated during watermelon cultivation and after processing

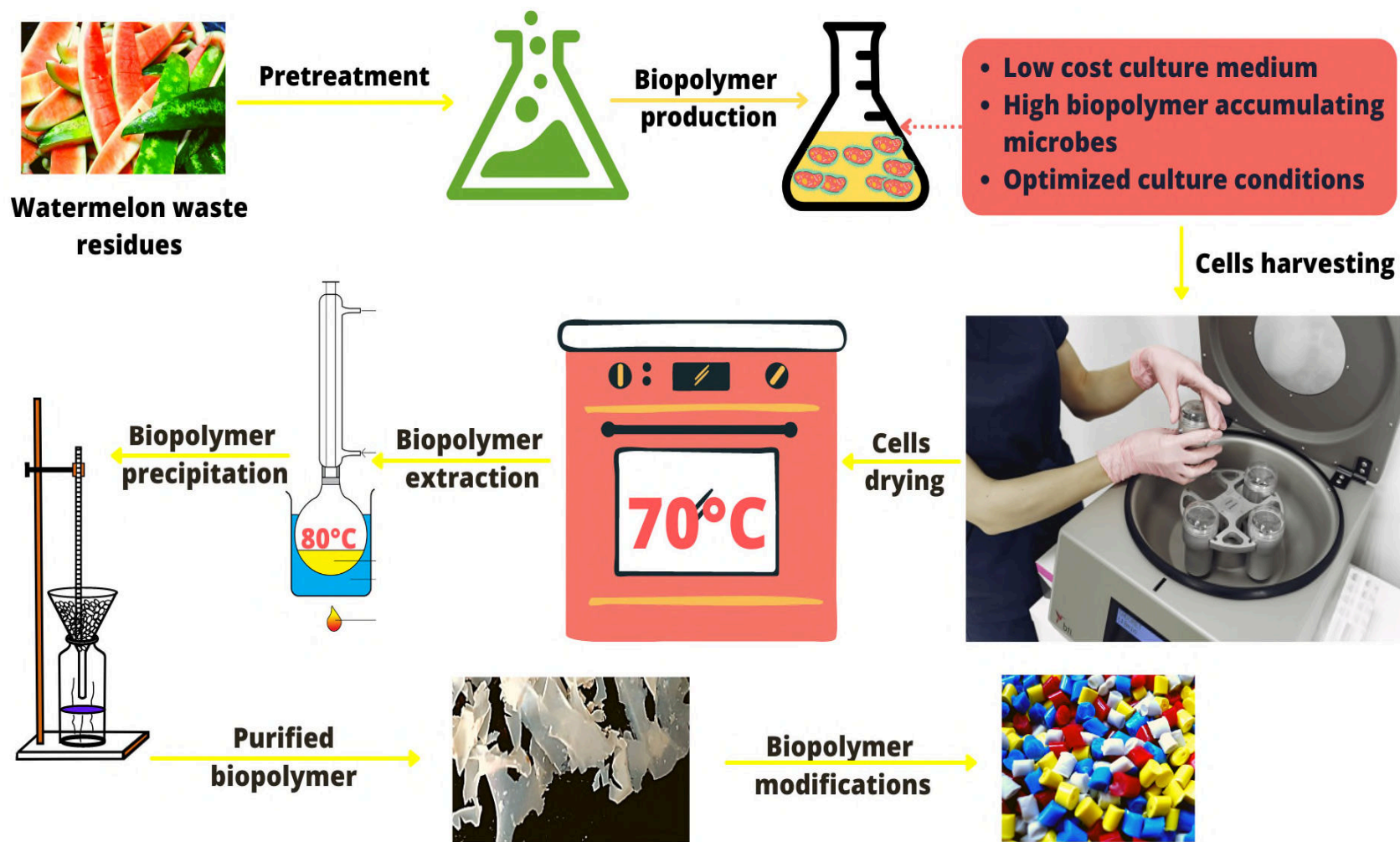


Fig. 3. Schematic presentation for production of biopolymer from utilizing watermelon waste residues

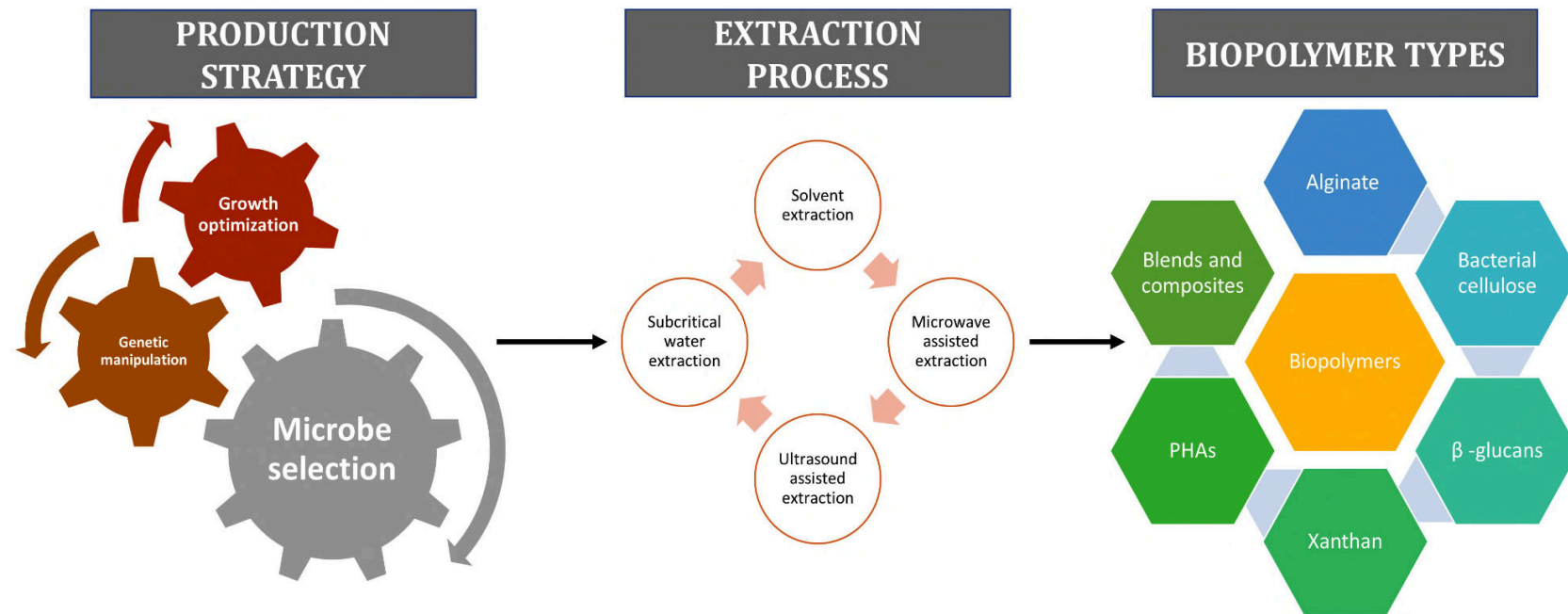


Fig. 4. Biotechnological strategies involved in the production of biopolymers.

Table 1: Proximate composition of seed and peel waste of watermelon.

Watermelon (<i>Citrullus lanatus</i>)			
Component (g/100g d.w.)	Seed	Peel	References
Moisture %	7.7-25	92-94	
Ash	2.3-3.8	3.2-18	Bakshi and Wadhwa, 2013;
Protein	18-25	7.4-18	Egbuonu, 2015;
Lipids	24-58	1.1-2.6	Morais et al., 2017;
Crude Fibre	2.5-49	3.1-93	Petkowicz et al., 2017;
Total Dietary Fibre		24-46	
Carbohydrates	13-29	28-85	
Pectin		19-26	
Cellulose		26	
Hemicellulose		0.1	
Lignin			

Table 2: Biopolymers types and their applications.

Biopolymer	Applications	References
Alginate	Biomedicine and food.	(Devadas et al., 2021 , Kartik et al., 2021)
Bacterial cellulose	Tissue engineering and wound dressing.	(Horue et al., 2021)
β -glucans	Cancer therapy and drug delivery.	(Yuan et al., 2020)
Xanthan	Food and industry raw material.	(Patel et al., 2020)
Polyhydroxyalkanoates	Biomedical applications and 3D printing.	(Kalia et al., 2021)
Biopolymer blends and composites	Tissue engineering and drug delivery.	(Horue et al., 2021)
Biopolymer hybrid systems	Tissue engineering and drug delivery.	(Horue et al., 2021)
PHAs blends and composites	Biomedical applications and drug delivery.	(De Donno Novelli et al., 2021)

Table 3: Various PHAs composites used in additive manufacturing or 3D printing.

PHA composite	Properties	Applications	References
Poly-3-hydroxybutyrate/lignin	High storage modulus and shear viscosity.	Wood industry	(Vaidya et al., 2019)
poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) /Fibrillated nanocellulose	High elastic modulus and yielding stress	Medical applications	(Valentini et al., 2019)
Polylactic acid/polyhydroxy butyrate/nanocellulose	High storage modulus	Bio-based products	(Frone et al., 2020)
poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)	Good storage modulus	Medical devices	(Giubilini et al., 2020)
Polyhydroxy butyrate/polylactic acid	Tensile strength (40-44MPa)	Biomedical applications	(Kontárová et al., 2020)
Polylactic acid/polyhydroxyalkanoates	Improved properties	Biomedical applications	(Rydz et al., 2020)
Wood flour/polyhydroxyalkanoates	Increased tensile strength and flexibility	Packaging industry	(Tian et al., 2021)

poly (3-hydroxybutyrate-co-3-hydroxyvalerate)/regenerated silk	Maximum loading	Piezoelectric device	(Bon et al., 2021)
Poly (3-hydroxybutyrate-co-3-hydroxyexanoate)	Tensile strength and yielding stress	Scaffold preparation	(Bon et al., 2021)

Production of watermelon



Physical

Chemical

Biological

Extraction and Purification

Cellulose



Hemicellulose

Thermochemical

Biological (Fermentation)

Production Process

Biopolymers

Organic acids

Bioenergy

Bioproducts

Phenolic compounds

Applications

Highlights

- Watermelon waste residues are a valuable and highly promising substrate for PHA;
- Microorganisms can synthesize biopolymers from watermelon waste residues;
- A high sugar content in the waste material is critical for competitive production yields; and
- The use of organic waste materials promotes a circular bioeconomy.

Current state of the art biotechnological strategies for conversion of watermelon wastes residues to biopolymers production: a review

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