REVIEW

Recent Advances in Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate) Biocomposites in Sustainable Packaging Applications

Rahul Dev Bairwan | Esam Bashir Yahya | Deepu Gopakumar

1Bioresource Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Penang 11800, Malaysia
2Bioprocess Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Penang 11800, Malaysia
3Green Biopolymer, Coatings and Packaging Cluster, School of Industrial Technology, Universiti Sains Malaysia, Penang 11800, Malaysia
4Department of Polymer Science & Rubber Technology, Cochin University of Science and Technology, Cochin 682022, India

*Corresponding author:
E-mail: akhalilhps@gmail.com
Tel.: +6046532219

ABSTRACT

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is the most promising and appropriate microbial biopolymer as a replacement for conventional petroleum-based non-biodegradable polymers, due to its excellent biodegradability and biocompatibility. However, it has a few limitations that prevent it from being used commercially, including low mechanical strength, hydrophobicity, poor thermal and electrical properties, difficult processing, and high cost. Recent research has shown that it is the most promising natural biopolymer, particularly for packaging. To use PHBV in biocomposites, methods of compensating for PHBV’s shortcomings, such as adding fillers, more cost-effective and efficient production methods, or alternative PHBV sources, must be developed. Numerous researchers are looking into ways to improve characteristics and lower prices by developing biocomposites to address environmental safety concerns with PHBV, developing and discovering more affordable biological PHBV production methods, discovering new microbial strains or strain combinations, or developing less expensive PHBV extraction methods. The current review provides a detailed description of the studies conducted to improve the properties of PHBV as biocomposites by employing less expensive yet efficient reinforcements, particularly for food packaging applications. Furthermore, nanocellulose can be studied further as a PHBV biocomposites enhancement to improve properties and functionalities from various optimal sources in order to produce fully degradable bionanocomposites for sustainable packaging applications.

KEYWORDS

PHBV Poly(3-hydroxybutyrate-co-3-hydroxyvalerate), biopolymers, biocomposites, nanocellulose, sustainable packaging.

INTRODUCTION

Environmental concerns have been in the spotlight for several decades, and the use of non-biodegradable plastics is increasing further day by day. It is primarily due to consumer waste disposal of packaging materials, which pollutes the environment, and it has become a challenge for researchers to control the usages of these non-biodegradable materials by replacing them with eco-friendly and degradable materials. Furthermore, the accumulation of non-biodegradable plastic material debris in land and oceans is harmful to the planet's health and contributes significantly to global warming and is majorly originating from packaging industry [1], with nearly 66% originating in the food packaging industries [2], shown in Fig. 1. To address all of these issues, a well-known solution is to use green sustainable materials or biopolymers that are biodegradable and biocompatible in nature. Globally, researchers are interested in working on sustainable biopolymers and biodegradable materials derived from natural resources, which offers significant advantages for biocompatible materials to be used as packaging materials for basic household items such as bottles, cans, toys, groceries, and utensils [3], and thus biopolymers are researched as potential substitutes for plastics derived from petrochemical industries.
Moreover in line of biopolymers, microbial biopolymers are the new generation materials which can be produced either by direct extraction from biomass or by bacterial fermentation offer good rate of degradability and renewability [4,5]. Polyhydroxyalkanoates (PHAs) are the most researched, produced, and used microbial polymers in the world. PHAs have significant biodegradability, biocompatibility, and renewability advantages over traditional petroleum-based synthetic polymers poly(ethylene and propylene). As a result, they can easily replace traditional polymers. PHAs are also excellent sustainable replacements because they are aliphatic microbial polyesters that are naturally synthesized by various microorganisms [6,7]. In comparison to PLA, the PHA family is completely biodegradable, making it appealing for commercialization. Based on the number of building blocks, PHA families are further classified as homopolymers or copolymers.

Poly (3-hydroxybutyrate) is the most well-known and researched variant of the PHA family (PHB). PHB is a homopolymer (one single monomer as the building block) that has received a lot of attention for its applications in biomedicals, tissue engineering [8], packaging, biodegradable bags [9], and so on [10]. However, its commercialization does not appear to be a perfect replacement for conventional plastics such as polypropylene because PHB is extremely brittle, expensive, has a limited processing window, and suffers thermal degradation due to poor thermal stability [11,12]. To overcome these disadvantages, several PHB copolymers are naturally synthesized, including poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV. PHBV has been found to be the most promising, thoroughly studied biopolymer, attracting the attention of researchers and industrialists due to its excellent eco-friendly properties. It was developed primarily to address the drawbacks associated with PHB. PHBV is more ductile than PHB and has good processability at lower temperatures, which reduces the problem of thermal degradation [11]. The properties of PHBV actually depends on the ratio of monomers in it, where 3HB contributes to stiffness and 3HV promotes flexibility. PHBV is actually produced by adding 3HV monomer in PHB(Polyhydroxybutyrate). PHBV is a transparent thermoplastic and an aliphatic polyester that is naturally produced by microorganisms and undergoes bacterial degradation [13]. It is well known for its excellent biodegradability, biocompatibility, and renewability, as well as its easily adjustable mechanical properties [14,15]. PHBV, like PHB, is produced through a fermentative process, with the exception that PHBV fermentation uses propionic acid with glucose as a carbon source, and variation in propionic acid percentage controls the (hydroxyvalerate) HV content in the produced copolymer [16] with the added benefit of biocompatibility and biodegradability [17]. PHBV has acceptable water vapour and oxygen transfer properties, liquid viscosity, is chemically inactive, and is resistant to ultraviolet radiation, making it suitable for packaging [3,18]. PHBV has a high degradability profile and can certainly degrade in natural environments such as soil and marine waters [19].

Despite the fact that PHBV has excellent eco-friendliness, it does have some drawbacks that limit its use in certain industrial applications. Low crystallinity, large crystal size, slow crystallization rate, low barrier properties, low impact strength, and a small processing window characterize PHBV [20,21]. Furthermore, the cost of PHBV and its production is quite high, so it cannot be used for mass production of everyday usable products [22] and global distribution [23]. To overcome PHBV’s limitations, various methods for improving properties and performance have been investigated, shown in Fig. 2. The most basic and effective method is to increase the HV content, as low HV content results in a loss of flexibility, impact strength and elongation at break [24]. Increasing HV content improves biodegradability, functional properties, processability, toughness, and elongation at break while decreasing crystallinity and melting temperature [16,19]. According to one study, adding nucleating agents such as thymine and melamine has accelerated the crystallization rate, increased the melting temperature of PHBV, and improved overall mechanical properties [25]. To deal with the higher costs of PHBV, which are heavily dependent on process conditions [26] for packaging and biomedical applications, one option is to produce it from a cheap carbon source [27,28], or to research effective microbial strains that can produce highly efficient polymer [29], or to use it with some cheap and effective natural fibre fillers [30,31], or to blend it with other biopolymers and use it as in biocomposites [32,33], which results in lower production costs and cost of ownership [34]. PHBV biocomposites with natural fibres as reinforcements [35,36] have received a lot of attention as a great replacement for synthetic fibres in industrial applications because natural fibres have eco-friendly properties, optimum mechanical strength, low weight, good insulating characteristics, renewability, and low costs [37,38]. Reinforcing agents such as kenaf, wood fibre, wheat straw, chitosan, bamboo pulp fibre, and cellulose fibre are used to improve the properties of PHBV [15]. Nano fillers (cellulose nanocrystals (CNCs)) have also been found to be suitable for the formation of bionanocomposites due to their low weight, availability, high strength, high surface area to
volume ratio, and ability to produce tough and stiff biocomposites. Due to its exceptional properties, it has recently gained interest in biomedical applications for drug delivery [39] and rigid packaging [37]. Aside from biomedical, it can be found in everyday disposables such as packaging, containers, cosmetics, bags, hygiene products, helmets, and car panels [10].

**IMPROVED PRODUCTION OF PHBV**

PHBV (3-hydroxybutyrate-co-3-hydroxyvalerate) is a thermoplastic copolymer of PHB (polyhydroxybutyrate) that can be synthesised from a variety of microorganisms and is primarily synthesised under limiting accumulating conditions [41]. The synthesis of C4 and C5 monomers, 3-hydroxybutyrate and 3-hydroxyvalerate (3HV), occurs during the production of PHBV [42]. A variety of gram positive and gram negative bacteria can synthesize PHBV [43]. Purification of PHBV synthesised naturally from gram positive bacteria genes (Nocardi on or Rhodococcus) is difficult and thus considered uneconomic [44]. Furthermore, PHBV uses recombinant glucose and propionic acid [45] with propionic acid serving as a precursor for 3HV and the amount of propionic acid influencing the synthesis process and 3H fraction. Unfortunately, high levels of propionic acid are toxic to bacteria cells, and the fractions of 3HV produced in copolymers are low [46]. Furthermore, PHBV synthesised by gram negative bacteria contains endotoxins, limiting its use in medical applications. Thus, PHBV requires purification from both types of bacterial genes, which raises the production cost and limits mass production [47].

Various production methods have been investigated in order to produce effective and cost-effective PHBV. The main aspects of production that must be considered are substrate type, microorganism (or combination of substrate and microorganism), and processing conditions [26]. According to research, 3HV content in PHBV is highly responsible for the enhancement of PHBV properties, so it is important to use 3HV precursors to improve 3HV fraction in PHBV biopolymer, but using 3HV precursors also increases production cost, which remains a barrier in its global production [34].

To address all of these issues and produce PHBV with a high 3HV fraction, various microbial strains and bacterial species have been investigated as carbon sources to replace the use of expensive 3HV precursors, and serve as a valuable way in reducing the cost of PHBV production and enhancing 3HV fraction control, as *Escherichia coli* [44, 48] or by using combinations of bacterial strains and organic waste as substrate can also favour reduction in PHBV production cost [34]. Through metabolic engineering techniques, some bacterial species can be modified or engineered to produce PHBV and increase productivity [49, 50]. A current and future production enhancing scenario of PHBV through all these strategies is shown in Fig. 3 [34]. Furthermore, 3HV content influences mechanical and physical properties, reduces crystallinity, and improves biodegradability [43].
produce PHBV with propionate as an additional carbon source [56,58]. PHBV as a copolymer uses propionate as a carbon source, which produces 3HV precursor, raising the production cost. Precursors are generally expensive and should be avoided. They are also detrimental to cell growth [59,60]. The primary goal of PHBV research is to reduce production costs, primarily by not using precursors, and to improve mechanical properties, which can be improved by increasing the 3HV fraction. PHBV can be produced by a variety of bacteria using carbon sources such as fatty acids, carbohydrates, glycerol, and so on, depending on the host [61]. To reduce production costs, open mixed cultures are being reported to produce PHBV, which uses a variety of cheap or waste feedstocks and does not require sterile conditions [59,62]. The archaea Haloferax Mediterranei has recently attracted many researchers due to the requirement for high quality PHBV production and increased productivity [63]. Its growth rate is much faster in comparison, and by avoiding sterile conditions, it reduces production costs [64,65]. Most importantly, Haloferax Mediterranei can degrade substrates without any pretreatments and produce a high amount of PHBV from low-cost feedstocks such as waste glycerol [66] and olive mill wastewater (OMW) [27]. In one report, Haloferax Mediterranei first produced PHBV using stillage and later explained that Haloferax Mediterranei requires excess salt, which must be disposed of after use and can be reused [67].

Several other studies have been published on the production of PHBV from various bacterial strains with increasing 3HV fraction. PHBV with 15% mol 3HV unit was synthesized in the lab from the bacterial strain Bacillus cereus FA11 via a shake flask fermentation experiment [68]. Another study found that Halogranum amylolyticum TNN58, an extreme halophilic archaeal strain, produced PHBV with a 20 mol% 3HV content [69]. A study reported for the first time the production of PHBV from the bacterial strain Bacillus aryabhattai PHB10 in the presence of propionic acid, for the preparation of PHBV-polyethylene glycol blends for skin graft applications [70]. Several novel bacterial strains were discovered that can grow faster and produce PHBV in greater quantities from carbon sources other than sugars. Pure bacteria, on the other hand, will necessitate sterile conditions and feedstocks, raising the overall production cost. As a result, researchers are now concentrating their efforts on locating low-cost or renewable feedstocks [71]. Haloferax mediterranei, a halophilic archae, can produce PHBV from unrelated carbon sources, yielding higher yields and lowering production costs by using waste as feedstock. Carbon sources account for nearly 40% of total PHA operating costs, so it is preferable to find cost-effective ways to produce this polymer from low-cost raw materials or waste feedstocks [72]. Haloferax mediterranei produces PHBV with a 55% higher yield than glucose by using food waste as a low-cost feedstock. It was discovered that short chain carboxylates, which are also important carbon sources, produced PHBV with a higher yield than glucose [73]. Using waste as a feedstock, not only lowers production costs but also aids in the management of waste from landfills and the prevention of environmental pollution. Similarly, among 96 types of activated bacterial strains capable of producing PHA in wheat starch wastewater (WSW). Bacillus cereus, a wild strain, demonstrated the ability to produce cost-effective PHBV biopolymer using WSW as an efficient substrate. Bacillus cereus PHBV nanoparticles of ~136 nm (diameter) confirmed optimal size and acceptable zeta potential [74]. Unrelated carbon sources are being studied extensively these days because they provide an efficient and cost-effective method of producing PHBV, shown in Fig. 4. However, it is now well understood that a significant amount of PHBV with high 3HV constituents can be synthesised naturally from a variety of bacterial strains. However, they require precursors, which are associated with higher production costs. Rhodospirillum rubrum, a bacterial strain used in a two-step PHBV production strategy, produced 46.5 mol% of PHBV using fructose as a carbon source. PHBV exhibited excellent mechanical properties, thermal stability, and low molecular distributions [75].

![Image](https://example.com/image.png)

**Fig. 4.** Overcoming the low yield and high production cost limitation of PHBV, effectively synthesizing it from various waste sources.

Volatile fatty acids (VFAs) found in wastewater or anaerobic organic wastes are another unrelated carbon source used to grow bacterial strains. VFAs contain nutrients that serve as a source of energy and are regarded as ideal feedstocks for the production of low-cost PHBV [76]. According to one study, three different bacterial strains *M. nitratireducens*, *M. zhoushanense* and *M. Sediminicola* was studied for its ability to produce PHA from volatile fatty acids and sugars. The findings revealed that *M. sediminicola*, a halophilic bacterium, demonstrated potential PHBV production with ~50 mol% 3HV fraction, which can serve as a unique platform for metabolic engineering techniques for PHBV production [77]. Another study discovered a new gram-negative strain of Photobacterium sp. TLY01 of Photobacterium species from marine environments was discovered to be a promising producer, producing the highest PHBV titers by
using waste substrates such as cooking oil, soyabean oil, and corn starch [78]. There is still the possibility of discovering several novelier bacterial strains in various waste feedstocks, both aerobic and anaerobic, capable of producing PHBV of high quality, with a high 3HV fraction, and at much lower costs.

From various substrates

Another strategy for increasing the productivity of PHBV, either in terms of lowering production costs or increasing quality, is to choose an appropriate substrate, as it also plays an important role in PHBV production. Researchers have already examined and discussed various aspects of production substrates. Using any feedstock as a substrate should serve as a carbon and energy source for the production of PHBV. Feedstock as substrate has potentially shifted from lignocellulosic substrates to renewal organic wastes, potentially significantly lowering the cost of PHBV production. As a result, many researchers are concentrating their efforts on developing low-cost and renewable feedstocks [79].

Lignocellulose, a low-cost and renewable agro waste feedstock, is now used as a co-substrate precursor in PHBV production alongside propionate, valerate, methane, and others [80,81]. Though propionate and valerate are toxic, expensive, and can inhibit microbial cell growth [82], they are still used in small amounts as precursors to potentially and economically produce PHBV because they can change the 3HV content and improve the properties of the PHBV produced. For the first time, lignocellulosic hydrolysate and acetate are used as lignocellulosic hydrolysate and acetate as precursors for the low-cost synthesis of PHBV [83,84].

Several studies have used propionic acid and valeric acid as precursors and have increased the 3HV fraction to a maximum while also improving polymer quality. Imperial Chemical Industries Limited used propionic acid for the first time in the production of PHBV in 1970. Following this, several researchers used propionic acid as a precursor mixed in culture medium with various strains, such as Ralstonia eutropha H16 [85], Bacillus thuringiensis R-510 [86]. It was quickly discovered that without a precursor, microbial strains could only produce PHB. The addition of propionic acid as a co-substrate increased the 3HV fraction and improved the polymer's mechanical and thermal properties [69]. A team produced PHBV with a 13.3% 3HV fraction that has improved thermal stability, melting temperature, and tensile strength. When 100% valeric acid was used as a precursor in the culture medium for the bacterium Ralstonia eutropha, 62% 3HV fraction in PHBV was obtained. Aside from that, there is a decrease in the glass transition temperature and melting temperature of PHBV, as well as a high amount of precursor leads to bacteria inhibition [87]. When valeric acid was used in a high sugared culture medium, Caldimonas taiwanensis, a bacterial strain, produced PHBV with increased 3HV content from 10% to 90% [88]. Both precursors, when used as co-substrates, are capable of producing high 3HV fractions in PHBV. The disadvantages of these precursors are that they are toxic, inhibit bacterial growth when used in large quantities, also are quite expensive, which ultimately raises the cost of producing PHBV. Propionic acid is actually more toxic than valeric acid. In light of this, several low-cost compounds were tested. Pentanol, for example, was tested and found to help increase valerate percentage and produce higher quality PHBV. It is, however, less effective than valerate and toxic to bacterial strains [89]. Pentanol and methane mixtures were found to be less effective than valerate and methane mixtures in the production of PHBV from the bacterial strain Methyllocystis WRRC1 [90]. In any case, researchers discovered a chemical compound, levulinic acid, which was the least expensive of all. Selecting the right substrate for production can significantly reduce production costs, as carbon substrate costs account for 28% to 50% of total production costs [91]. Ralstonia eutropha tested levulinic acid as a co-substrate precursor with primary carbon source fructose for producing PHBV and found that it increased the 3HV fraction from 7% to 75.1% [52]. Similarly, Ralstonia eutropha is used in conjunction with levulinic acid to produce a cost-effective PHBV biopolymer as well as to improve the thermal properties of PHBV. Because levulinic acid is responsible for producing and increasing 3HV fraction, PHBV with 53.9% 3HV fraction was produced, with improved flexibility, ductility, and workability. One minor finding was that the C:N (carbon and nitrogen) ratio and stress resistance conditions play an important role in obtaining proper 3HV content [92].

Conclusively, after several researches a fascinating alternate came up of completely replacing precursors by using waste substrates as an unrelated carbon source to sharply reduce the production costs. Various microbial strains showed potential to produce PHBV with wastes and even without precursors and subsequently have achieved high 3HV fractions. Strains like Bacillus cereus [93], Bacillus Flexus [94], Yangia ND199 [95], Bacillus OU40T [96], Pseudomonas aeruginosa [97], PNSB Rhodospirillum Rubrum without precursors produced PHBV with 46.5% 3HV content, highest from unrelated carbon sources [75]. There are many compounds present in wastes from the oil industry, agro wastes [98], food wastes, waste water, and so on, such as glucose, maltose, glycerol, and various other chemical compounds, which may help bacteria accumulate and serve as a great source of carbon and energy to produce PHBV. Most organic wastes contain cellulose, carbohydrates, proteins, and lipids, which provide a medium of volatile fatty acids for bacteria like Ralstonia eutropha to produce PHB or PHBV after fermentation. PHBV was successfully synthesised two decades ago by Ralstonia eutropha under oxygen limitation culture environment using two fermentation methods using industrial waste and vegetable & fruit waste and was compared to commercial glucose-based PHBV. Organic wastes produced PHBV of 30/70 mol% 3-HV to 3-HB, confirming that they are acceptable carbon sources.
However, oxygen limitation reduces PHBV production, but it was considered the only approach to processing organic wastes. Since then, several studies have been conducted to reduce the cost of PHBV production and to discover the best nutritional environment for bacteria to produce economical and high-quality PHBV [99].

![PHBV Production with precursors as substrates](image)

**Fig. 5.** Precursor based PHBV production are costly and harmful whereas without precursor PHBV production from waste substrates (including pretreatments) is always less expensive and environmentally favourable, pictures adapted from waste substrates [100], waste water [101], agro waste [102] and waste oil [103].

Organic wastes and wastewater can be used as substrates to reduce production costs and are a promising strategy for producing PHBV. For example, crude glycerol, one of the most cost-effective and widely used substrates as industrial waste from biodiesel industries [94,104], is ideal for the production of PHAs because the carbon atoms in glycerol are very strong and promote biopolymer synthesis. Crude glycerol produces PHBV from *Haloferax mediterranei* with the same quality and properties as pure glycerol, but is considered more suitable due to its lower cost and availability [66]. In comparison to glucose, fructose, maltose, sucrose, xylose, and dextrin, glycerol produces the highest quality PHBV with a high 3HV fraction [94]. Another economic substrate is byproducts from ethanol industries, which contain sufficient nutrients for the accumulation of a variety of bacteria species for the production of PHBV. *Bacterium Rhodospirillum Rubrum* promotes PHBV production in a condensed corn soluble (CCS) medium, a byproduct of the ethanol industry that contains enough organic acids as nutrients for bacteria accumulation, and is a cost-effective method of producing PHBV on a commercial scale [105]. Vinasse, a highly polluting waste from the ethanol industry, is pretreated on inexpensive activated charcoal and studied for the production of PHBV by *Haloferax mediterranei*. The results showed that PHBV could be produced with 12.36 mol% 3 HV (using 25% pretreated vinasse) and 14.09 mol% 3HV (using 50% pretreated vinasse) [106]. Then, *Haloferax mediterranei* tests stillage, another waste from the rice-based ethanol industry, and produces PHBV with 15.4 mol% 3HV fraction [67]. Both studies above confirmed that both substrates were potentially suitable for producing low-cost PHBV. Waste substrates necessitate some pretreatment, but they are always less expensive to process than other substrates, shown in Fig. 5.

Another efficient organic waste source is agro waste, which is low in cost and highly nutritional (sugars, proteins, and various organic acids) for bacterial accumulation and the production of PHBV. Rice straw is a competing candidate for the production of PHBV when compared to glucose and starch [96], rice bran and wheat bran as substrates [107], Indian Madhuca flowers [108], poplar hydrolysate [109], and so on.

In addition, wastes from food industries, such as waste water, vegetable oils, and organic wastes, are regarded as suitable and inexpensive substrates for PHBV production because they contain high amounts of sugar and other nutrients for the accumulation of a diverse range of bacterial species. Fruit and vegetable waste (FWV), typically the indigestible part, is a waste from these industries used for PHBV production, such as olive mill wastewater, a polluting waste from the olive oil industry, is used to produce PHBV by *Haloferax mediterranei*, resulting in the production of PHBV with a 6.5 mol% 3HV fraction. The interesting part was that PHBV produced with OMW demonstrated excellent bacterial cell growth without any fermentation step and with OMW as the sole carbon source [27]. *Cupriavidus necator* H16 bacteria used Jatropha oil as the main carbon source to produce PHBV; the results showed that the PHBV produced was of the same quality as PHBV produced from sugars and other plant oils as carbon sources. Jatropha oil is a promising and renewable carbon source that can be used to make PHBV commercially [54]. Another waste utilized is from dairy industry, cheese whey, as highly saline substrate is used for production of PHBV by *Haloferax mediterranei*, as it requires salt to cultivate. It could be very promising as it offers synthesis of PHBV copolymer by directly contacting biomass with water [110]. Similarly, cheese whey permeate, which is high in lactose, is used as the sole carbon source to produce PHBV from *Bacillus megaterium* [111]. Similarly, date palm crop waste from the Middle East region is used as a substrate to accumulate *H. mediterranei*, and the production of PHBV resulted in PHBV with an 18 mol% 3HV content [28]. This could be summarized as using organic wastes as a substrate for producing PHBV not only reduces the cost of production but also recycles the waste, reducing environmental hazards and pollution. However, waste substrates typically require suitable biological or chemical pretreatments, as well as mechanical and thermal treatment, to reduce solid waste size, extract nutrients, remove toxic compounds from waste, change the
production rate, and improve substrate efficiency [112]. Chemical pretreatments are less expensive than enzymatic pretreatments and are appropriate for the strain *Haloferax mediterranei* [110]. Biological anaerobic pretreatments are environmentally friendly and cost-effective, producing organic acids such as propionic, acetic, and lactic acid, which can then be used to synthesize PHBV [113]. This method was first used in 2002, when PHBV production was coupled with anaerobic fermentation of food waste in an anaerobic reactor [114]. Later, anaerobic fermentation of palm oil mill effluent waste water was used, and PHBV production from strain *Comamonas sp* EB 172 was confirmed [115]. Although pretreatments are required for waste substrates, the focus should be on toxic-free substrates that are rich in nutrients. Short summary of waste substrates based PHBV is discussed in Table 1 below.

**Table 1. PHBV from waste substrates.**

<table>
<thead>
<tr>
<th>Waste substrates</th>
<th>Bacterial strain</th>
<th>PHBV Production</th>
<th>Remarks for PHBV produced</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat stalk wastewater</td>
<td><em>Bacillus cereus</em></td>
<td>Concentration = 3.07 g/L; M.f. = 59.50% DCW; 3HV f. = 69.1% 3HV</td>
<td>PHBV nanoparticles; High zeta potential; Low cost production</td>
<td>[74]</td>
</tr>
<tr>
<td>Carbon dioxide or a simple organic substrate</td>
<td><em>Nostoc microscopicum</em></td>
<td>Concentration = NDR; M.f. = NDR; 3HV f. = 96.2% 3HV</td>
<td>High range of HV fraction; Cost effective</td>
<td>[116]</td>
</tr>
<tr>
<td>Madhuca indica flower extract</td>
<td><em>Ralstonia eutropha</em></td>
<td>Concentration = 1.44 g/L; M.f. = 49.4% DCW; 3HV f. = 27.8% 3HV</td>
<td>Excellent biodegradability; Corrosion resistance</td>
<td>[108]</td>
</tr>
<tr>
<td>Wastewater from biodiesel industry</td>
<td><em>Pseudomonas mendocina</em></td>
<td>Concentration = NDR; M.f. = 43.6% DCW; 3HV f. = 8.6% 3HV</td>
<td>Low-cost process</td>
<td>[104]</td>
</tr>
<tr>
<td>Crop waste from date palm fruit</td>
<td><em>Haloferax Mediterranei</em></td>
<td>Concentration = 18 g/L; M.f. = 25% DCW; 3HV f. = 18% 3HV</td>
<td>Amorphous nature increased degradation rates; Enhanced workability.</td>
<td>[28]</td>
</tr>
<tr>
<td>Rice straw</td>
<td><em>Ralstonia eutropha</em></td>
<td>Concentration = 0.5–3.9 g/L; M.f. = 39% DCW; 3HV f. = NDR</td>
<td>Heating time increase 3HV fraction and can reduce polymer productivity. Repeated hydrolysate feeding increases the 3HV content.</td>
<td>[117]</td>
</tr>
<tr>
<td>Cheese whey permeate</td>
<td><em>Bacillus megaterium</em></td>
<td>Concentration = 3.64 g/L; M.f. = 86.6% DCW; 3HV f. = 16.6% 3HV</td>
<td>PHBV with good tensile strength; Thermally stable between 100–400 °C.</td>
<td>[111]</td>
</tr>
<tr>
<td>Olive mill wastewater</td>
<td><em>Haloferax Mediterranei</em></td>
<td>Concentration = 0.2 g/L; M.f. = 43% DCW; 3HV f. = 6.5% 3HV</td>
<td>PHBV produced without fermentation or external carbon source. Low production cost</td>
<td>[27]</td>
</tr>
<tr>
<td>Cheese whey</td>
<td><em>Haloferax Mediterranei</em></td>
<td>Concentration = 7.9 g/L; M.f. = 54% DCW; 3HV f. = 1.5% 3HV</td>
<td>PHBV with a high molecular weight and low crystallinity; High salinity culture requirements; require strict sterility.</td>
<td>[110]</td>
</tr>
<tr>
<td>Poplar; Activated sludge</td>
<td>NDR</td>
<td>Concentration = 637.6 mg/L; M.f. = NDR; 3HV f. = NDR</td>
<td>Pretreatment with hot water improves efficiency and yield.</td>
<td>[109]</td>
</tr>
<tr>
<td>Whey and ricotta cheese exhausted whey (RCW)</td>
<td><em>Haloferax Mediterranei</em></td>
<td>Concentration = NDR; M.f. = NDR; 3HV f. = (1–3 mol%)</td>
<td>A sustainable carbon source for the large-scale production of PHBV</td>
<td>[63]</td>
</tr>
</tbody>
</table>

*DCW = Dry cell weight (g); 3HV f. = fraction of HV in PHBV molecule (m/m); M.f. = fraction of PHBV in cell or PHA (m/m); NDR = no data reported.

**From metabolic engineering strategies**

Metabolic engineering techniques, in which a variety of microorganisms and strains are metabolically engineered to produce biopolymers, are another approach to producing high quality PHBV and other biopolymers. It is about modifying cellular activities through the use of new DNA technology [118]. There are several case studies from the previous decade in which catabolizing sustainable substrates produced eco-friendly and effective biopolymers from various microbial strains and proved to be a successful way to improve polymer production. Among the various microbial hosts, *Escherichia coli* and *Saccharomyces cerevisiae* are the most common and well-known.

The main impediment to PHBV production was a lack of propionyl-CoA in bacterial strains, so expensive precursors were frequently used to produce high-quality PHBV. Thus, biosynthesis of PHBV with high 3HV content via metabolic engineering pathways derived from unrelated carbon sources, without the use of propionate precursors, may be the most cost-effective and efficient option. Aldor *et al.* previously synthesised PHBV with a 30 mol% 3HV fraction from an engineered *Salmonella enterica serovar Typhimurium* strain without the use of a propionate precursor. *E. coli* strains coli gene cloned in *Salmonella enterica*, where the recombinant successfully converted succinyl-CoA to propionyl-CoA and accumulated PHBV. Nonetheless, the procedure was expensive because it
required amino acids in the medium [119]. Another metabolic engineering study used threonine biosynthesis in *Escherichia coli* to demonstrate a more cost-effective pathway for producing PHBV from single unrelated carbon sources. There are several genetic variants of *E. coli* were created that were capable of producing PHBV with various 3HV fractions. The highest 3HV fraction found in PHBV was 17.5 mol%. This strategy aimed to produce low-cost PHBV while also making the process simple [120]. Other research has reported the production of PHBV from unrelated cheap carbon sources (glycerol and glucose), engineered *E. coli* strains were investigated, and biotechnological strategies (converting tricarboxylic acid (TCA) to propionyl-CoA) produced PHBV with a wide range of 3HV content ranging from 4 to 50 mol% [49].

Another microbial strains were also investigated through metabolic engineering techniques for the biosynthesis of PHBV and resulted in high 3HV fractions. *Corynebacterium glutamicum* strain WM001 isolated from soil was engineered with gene cluster *phaCAB*, the recombinant produced high pool of intracellular propionyl-CoA and then high levels of PHBV (72.5% 3HV fraction) and L-isoleucine (amino acid) to produce glucose as sole carbon source [121]. High 3HV fractions are required to enhance the mechanical and processing properties of PHBV. In view of which researchers try different recombinant to achieve high 3HV content in production of PHBV and for that propionyl-CoA is must. One research reported, new method to vary 3HV monomer composition by using propionyl-CoA transferase gene (pct) with PHA biosynthetic genes *bktB*, *phaB* and *phaC* from *Ralstonia eutropha* into genetically engineered *E. coli*. Recombinant PHBV was successfully produced at a high 80% wt. of 3HV content. This work suggested using Pct enzyme, for production of propionyl-CoA as 3HV precursor [122]. Similarly, in order to increase the 3HV content in PHBV, cloning of *prpE* gene encoding propionyl-CoA synthase, *vbg* gene encoding bacterial hemoglobin (VHb) and polyhydroxyalkanoates (PHAs) synthesis operon (*phatCAB*) in one plasmid is done and converted into *Escherichia coli* XL1-blue. The results revealed high 3HV content and high molecular weight of PHBV [123]. Aside from economic and quality PHBV production, researchers are attempting to increase the molecular weight and cell density of PHBV in order to increase production, as this aids in biomass recovery processing. Another metabolic engineered recombinant *Halomonas* TD01 from overexpression of threonine synthesis pathway and threonine dehydrogenase successfully produced PHBV with 4-6 mol% 3HV from carbohydrate as sole carbon source, revealing that recombinant lagre cells also enhanced polymer accumulation [124]. Amino acids such as threonine and isoleucine are already effective compounds in glucose for increasing the 3HV fraction in polymer. Choi et al. used metabolic engineering techniques to produce PHBV based on threonine biosynthesis, using a threonine overproducing mutant strain (MT-16) of *Alcaligenes sp. SH-69* derived from glucose substrate PHBV production with more than 22 mol% 3HV was six times higher than in the wild type strain under the same culture conditions [125].

The recombinants mentioned above use either plasmid expression or chromosome expression to produce PHBV. Plasmids have high efficiency of expression but require antibiotics for stability [126], whereas chromosomes have higher stability without antibiotics but lack strength [127].

The tricarboxylic acid (TCA) cycle, a suggested central carbon metabolism (CCM) pathway, provides an intermediate pathway where both cell growth and 3HV fraction can be improved. As a result, an attempt was made to engineer TCA cycles using novel chromosomal expression strategies and to create a recombinant *Halomonas bluephagenesis* TD01 strain using glucose as the sole carbon source. The strategies included weakening 3HV consumption pathways, increasing 3HV precursor synthesis flux, and activating the ED pathway to reduce the NADH/NAD+ ratio in order to improve TCA cycle activity. The engineered species was able to successfully produce PHBV with (0-25) mol% 3HV fractions. This research will lead to the development of next-generation industrial biotechnology (NGIB) to produce low-cost PHBV [128]. Another simple carboxylic acid, acetate used as an alternate carbon source to produce PHBV from metabolically engineered *Aeromonas hydrophila* 4AK4. Recombinant, overexpressed b-ketothiolase, acetoacetyl-CoA reductase and acetyl-CoA synthetase to successfully accumulate PHBV [50].

Nowadays, research is focusing on producing PHBV at a low cost, on a large scale, and with high yields. In this regard, microorganism selection is an important consideration. According to the authors, a developed bioprocess strategy employs natural PHBV producer *Bacillus megaterium* from unrelated carbon sources to produce PHBV from simple carbon source glucose without the addition of any precursor. B. *megaterium* NRRL B-14308 strain increased PHBV copolymer production with a high 3HV content of 58 mol% in fed-batch fermentations via *phaC* gene overexpression as recombinant [129]. Similarly, the haloarchaeon *Haloferax mediterranei* can produce PHBV from inexpensive substrates without the addition of 3HV precursors. Additional genetic manipulation is also required to improve cell growth. CRISPR (clustered regularly interspaced short palindromic repeats interference) genetic manipulation is being used to direct carbon flux from central metabolism (prime source) to improve PHBV accumulation, reduce fermentation periods, and increase overall biopolymer productivity [130]. PHBV can be produced in large quantities by *Haloferax mediterranei*. Around ten years ago, an intriguing strategy was used to improve PHBV production from *Haloferax mediterranei* by eliminating the gene cluster responsible for producing exopolysaccharide (EPS), a by-product. An EPS-mutant strain was created that eliminated EPS synthesis and used that portion of energy from carbon sources to produce PHBV; this strategy
successfully increased PHBV production by 20% when compared to the wild strain [131].

Formate, an eco-friendly low-cost C1 feedstock, is another promising cost-effective way to produce PHBV other than sugar-based feedstocks. Bacterium Methylorubrum extorquens gets its carbon from formate. M. extorquens strains that had been metabolically engineered produced PHBV with an 8.9% 3HV fraction. However, the addition of butyrate increased the 3HV fractions to 70.6%. The high proportion of 3HV obtained by using formate via metabolic engineering confirms its potential and can be investigated further to produce PHBV without the addition of precursors [132].

**PHBV WITH OTHER MATERIALS**

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV, microbial copolymer has received widespread attention due to its excellent biodegradability, nontoxicity, optical activity, and biocompatibility, making it useful in a variety of applications [133,134]. Researchers have attempted to improve the performance of PHBV through various means in recent years in order to overcome issues associated with neat PHBV such as brittleness and high cost [135,136]. Other materials, such as natural fibres, other polymers, nanomaterials, and so on, must be added to PHBV in order to use it efficiently and improve its performance.

### Table 2. PHBV reinforced natural fibre biocomposites.

<table>
<thead>
<tr>
<th>PHBV/ Natural Fibre</th>
<th>Fibre content (wt.%)</th>
<th>Processing Method</th>
<th>Influence in properties</th>
<th>Applications</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood fibres (WF)</td>
<td>7.5%</td>
<td>Injection molding</td>
<td>Enhancement of elasticity modulus, thermal stability, and water absorption</td>
<td>Biocomposite material</td>
<td>[24]</td>
</tr>
<tr>
<td>Basalt fibers (BF)</td>
<td>15%</td>
<td>Injection molding</td>
<td>Tensile strength, modulus of elasticity, impact strength, flexural strength, flexural modulus, thermal stability, and water absorption are all improved.</td>
<td>Biocomposite material</td>
<td>[24]</td>
</tr>
<tr>
<td>Wood fibre</td>
<td>(10, 20, 30, and 40)</td>
<td>Extrusion</td>
<td>Mechanical properties improve while thermal stability deteriorates</td>
<td>Biocomposite material</td>
<td>[140]</td>
</tr>
<tr>
<td>Unidirectional flax fibre and toughened with epoxidized natural rubber</td>
<td>NDR</td>
<td>Hot compression molding</td>
<td>Tensile, flexural, and impact properties are improved.</td>
<td>Green Composites</td>
<td>[141]</td>
</tr>
<tr>
<td>Miscanthus (Misc) fibres and distillers’ dried grains with solubles (DDGS)</td>
<td>(15 and 25 wt%)</td>
<td>Co-rotating twin screw</td>
<td>Improved marine biodegradability and mechanical and biodegradable properties</td>
<td>Sustainable, low-cost and lightweight plastic products</td>
<td>[142]</td>
</tr>
<tr>
<td>Flax, hemp and wood fibre</td>
<td>30%</td>
<td>Extrusion</td>
<td>Some mechanical properties improved, No significant improvement in properties with wood fibre.</td>
<td>Packaging industry</td>
<td>[143]</td>
</tr>
<tr>
<td>(Phalaris arundinacea and Lonicera japonica)</td>
<td>(5–20) % v/v</td>
<td>Compounding twin-screw extruder</td>
<td>Improved thermal stability, limited crystallisation, Decreased rigidity, and Lower produced cost</td>
<td>Semi-rigid packaging and lightweight auto body panels.</td>
<td>[144]</td>
</tr>
<tr>
<td>Luffa Fiber (untreated luffa fibers (ULF) and NaOH-H2O2 treated I)</td>
<td>(0, 30, 40, 50, 60, 70, and 80%)</td>
<td>Hot press forming</td>
<td>Water absorption rates increased bending strength increased. Composites’ moisture resistance has been improved. and good interfacial adhesion</td>
<td>Composite material</td>
<td>[145]</td>
</tr>
<tr>
<td>Alkali treated Flax and Hemp Fibers</td>
<td>15 wt%</td>
<td>Extrusion</td>
<td>Young's modulus, tensile strength, and hardness of hemp and flax fibre composites improved slightly.</td>
<td>Composite material</td>
<td>[146]</td>
</tr>
<tr>
<td>Wheat straw fibers (WSF)</td>
<td>(6, 10, 20, and 30 %)</td>
<td>Twin-screw extruder</td>
<td>Functional property enhancement Young's modulus reduced</td>
<td>Food contact materials for packaging applications</td>
<td>[147]</td>
</tr>
<tr>
<td>Nettle fibers (stems and leaves), pine cones flour, and walnut shells flour</td>
<td>15 wt%</td>
<td>Extrusion</td>
<td>Improved Young’s modulus, higher stiffness stability at elevated temperatures, increased rate of hydrolytic degradation, and lower cost</td>
<td>Single-use applications</td>
<td>[30]</td>
</tr>
</tbody>
</table>

* NDR = no data reported.
 Until recently, PHBV reinforced natural fibres primarily used short fibres, filler, and nanofibers. Continuous long natural fibres containing PHBV are still rare. Nonetheless, studies have shown that when compared to PHBV reinforced short fibre biocomposites, there is a significant increase in mechanical properties. Unfortunately, this increase is accompanied by significant fibre embrittlement, resulting in marginal reductions in elongation to break. To combat embrittlement, toughening agents are primarily added to the polymer matrix. For the first time, PHBV was reinforced with unidirectional flax fibre to prepare biocomposites, and toughening agents such as poly(butylene adipate-co-terephthalate) (PBAT) or epoxidized natural rubber (ENR) were added to control embrittlement, shown in Fig. 6. Unidirectional flax addition increased tensile properties by four times, flexural properties by three times, and impact properties by twenty times, with nominal change in thermal properties. The addition of PBAT had no discernible effect on thermal or mechanical properties. The addition of ENR, on the other hand, reduced the tensile modulus and flexural properties while significantly increasing impact strength, owing to the coarse particle size of ENR [141]. Another study found that PHBV reinforced flax fibres treated with alginic acid had higher biodegradability in compost than PHBV/untreated flax fibres and pure PHBV [37]. Similar was observed with PHBV reinforced NaOH-H₂O₂ treated luffa fibres compared to PHBV/untreated luffa fibres. TLF/PHBV had a higher flexural strength than ULF/PHBV. Furthermore, pretreatment significantly improved the bonding between fibres and PHBV, resulting in improved moisture resistance of composites [145].

Although PHBV is well-known for its potential functionalities and is in high demand in food packaging applications, its stability in food contact conditions has yet to be thoroughly investigated. For the first time, the ability of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)/wheat straw fibres (WSF) biocomposites as food contact materials for packaging applications was investigated. The findings revealed that water activity was the primary factor governing the overall migration of PHBV/WSF materials (a_w). As a result, such composite materials could only be used as food contact materials in foods with a water activity of 0.90 or less [147].

Several cellulosic fibres are also being investigated as PHBV reinforcement to strengthen its properties, and researchers are now experimenting with different approaches and techniques to improve the properties even further. Alkali-treated cellulosic fibres are more thermodynamically stable and have a high strength and thermal resistance. The mechanical, processing, and usable properties of PHBV biocomposites made by reinforcing alkali-treated hemp and flax fibres were studied. PHBV/alkali treated hemp biocomposites improved in all properties when treated with 10% NaOH solution, whereas PHBV/alkali treated flax biocomposites decreased in mechanical properties. The concentration of alkalizing solution should be chosen individually for each type of natural fibre and polymer matrix, according to the findings [146].

Furthermore, as PHBV reinforcement, several lignocellulosic fibres have been reported. Lignocellulosic fibres have a high stiffness, are light in weight, biodegradable, and, most importantly, are less expensive. Reusing agricultural and forest wastes, on the other hand, can reduce production costs and reliance on natural resources. Basalt fibre is also gaining popularity as a reinforcing material. Reinforced fibres from different sources (wood and basalt) were used to make PHBV biocomposites at 7.5wt% and 15wt%, respectively. Overall, the properties of PHBV/BF biocomposites improved. PHBV/WF biocomposites increased modulus of elasticity while decreasing strength by 10%. The primary concern with PHBV in terms of mechanical properties is its low impact strength, especially when compared to PLA. In contrast, PHBV-based composites are becoming competitive with PLA composites, particularly at high temperatures. Biocomposites of PHBV reinforced with 15% basalt fibre, on the other hand, increased impact strength by 41% and Young’s modulus by 74%. Furthermore, all of the prepared biocomposites were stiffer than pure PHBV [148].

PHBV/other biopolymer

One of the appropriate reinforcement strategies for addressing PHBV’s flaws is to mix it with other polymers. As a result, high-quality materials with improved mechanical properties, degradability, and immiscibility are produced. PHBV is commonly blended with poly (butylene adipate-co-terephthalate) PBAT to improve brittleness. Furthermore, the butylene group improves PHBV’s flexibility and impact strength. The tensile strength and storage modulus of PHBV/PBAT polymer blends increased, and a rare phenomenon of melting point elevation was observed [149]. Similarly, PHBV/PBAT polymer blends improved impact strength and elongation at break improved as PBAT and plasticizer content increased. The PHBV blend has the potential to be used in the packaging industry [150]. Some PHV blends with other biopolymers with and
without plasticizers are mentioned in Table 5. It is important to note that the addition of other compounds, such as plasticizers (e.g., polyethylene glycol and lauric acid), to PHBV films can reduce their stiffness and breaking strength when compared to pure PHBV films. Contrarily, brittle biomaterial hydrogels like poly(hydroxyethyl methacrylate) (PHEMA) can be strengthened with PHBV through photopolymerization of HEMA in the presence of PHBV to produce IPNs with improved mechanical characteristics [151].

Table 3. PHBV Blends with other biopolymers.

<table>
<thead>
<tr>
<th>PHBV/Blend (NP)</th>
<th>Weight percentage</th>
<th>Processing Method</th>
<th>Influence in properties</th>
<th>Applications</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV/thermoplastic polyurethane (TPU) using hexamethylene diisocyanate (HMDI)</td>
<td>PHBV 100/ TPU 30</td>
<td>Reactive extrusion</td>
<td>Improved ductility Small improvements in toughness creep resistance improved</td>
<td>Rigid packaging</td>
<td>[152]</td>
</tr>
<tr>
<td>PHBV/PCL/epoxidized palm oil (EPO)</td>
<td>PHBV 60/PCL40/1 EPO</td>
<td>Melt mixing</td>
<td>Strength, flexibility, and toughness improved.</td>
<td>Industrial applications</td>
<td>[153]</td>
</tr>
<tr>
<td>PHBV/PBS/halloysite nanotubes (HNT)</td>
<td>PHBV/PBS (80/20, 50/50 and 20/80)/5% HNT</td>
<td>Melt compounding</td>
<td>Improvement in fire reaction, but decrease in thermal stability</td>
<td>Nanocomposites</td>
<td>[154]</td>
</tr>
<tr>
<td>PHBV/natural rubber latex (NR)</td>
<td>PHBV/NR ratio 2:3, 1:1 and 3:2</td>
<td>Extrusion</td>
<td>Tensile strengths and elastic moduli are increased.</td>
<td>Packaging films</td>
<td>[155]</td>
</tr>
<tr>
<td>PLA/PHBV</td>
<td>varied from (10/90 to 90/10) in 10 wt.% increments</td>
<td>Melt mixing</td>
<td>Thermal conductivity has increased, but tensile strength and Young's modulus have decreased.</td>
<td>Packaging</td>
<td>[156]</td>
</tr>
<tr>
<td>PCL/PHBV</td>
<td>PCL/PHBV (1000, 75/25, 50/50, and 25/75)</td>
<td>Fused deposition modeling</td>
<td>Improved physical, mechanical, and biological properties of porous scaffolds with increased roughness, wettability, and hydrophilicity after plasma treatment</td>
<td>Cartilage tissue engineering applications</td>
<td>[157]</td>
</tr>
<tr>
<td>PHBV/PBAT</td>
<td>PHBV 70/ PBAT 30</td>
<td>Extrusion</td>
<td>Consistent mechanical properties Better functional properties</td>
<td>Food packaging</td>
<td>[158]</td>
</tr>
<tr>
<td>PHBV/PBAT with biodegradable plasticizer (citrate ester)</td>
<td>PHBV/PBAT (90/10, 80/20 and 70/30)</td>
<td>Melt compounding</td>
<td>Toughness and elongation increase while the Young's modulus and tensile strength decrease</td>
<td>Packaging industry</td>
<td>[150]</td>
</tr>
<tr>
<td>PHBV/BioPBS-blend (reinforced with talc and starch)</td>
<td>80% BioPBS/20%PHBV</td>
<td>Melt compounding/compression molding</td>
<td>Biodegradability, toughness, and tensile strength improved.</td>
<td>Single-use packaging</td>
<td>[17]</td>
</tr>
</tbody>
</table>

*PCL: Polycaprolactone, PBAT: Polybutylene adipate terephthalate, PBS: Polybutylene succinate, PLA: Polyactic Acid

Poly(lactic acid) (PLA), has been studied in several studies as suitable biopolymer for packaging applications [159,160] and as an effective way to improve polymer properties with PHBV. Despite the fact that PHBV and PLA are brittle and immiscible, PHBV/PLA blends result in improved ductile plastic deformation and thermal stability [161]. Furthermore, it has been reported that combining 20-35% PHBV with PLA is a suitable combination for achieving high barrier properties to both oxygen and water vapour while maintaining the material's biocompatibility [162]. Creating multilayers of different biopolymers in a single sheet can improve polymer properties while also overcoming polymer immiscibility caused by poor interfacial adhesion between phases of polymer mixtures [163]. PHBV and PLA were chosen for multilayer films, while thermoplastic chitosan (chemically derived from chitin) is used as a barrier layer to improve multilayer film adhesion and gas barrier properties [164]. PHBV was also used as a ternary composite with PLA and cellulose to achieve 100% biodegradability. Additionally, in the report, ball milling of pulp fibre was investigated experimentally and revealed that ball milling resulted in lower crystallinity and smaller particle size, which increased the stiffness of the ternary composite dramatically, confirming its potential for packaging applications [165]. In addition to PHBV/PLA, phenolic acids, p-coumaric, and protocatechuic) and a plasticizer are added as antibacterial compounds in PHBV/PLA blend films for active packaging applications [166].

Blending PHBV with starch, with low oxygen permeability, yields composite materials with improved mechanical properties and low water vapour permeability, making it more suitable for food conservation and packaging [167,168]. PHBV microparticles/potato starch films with added glycerol as a plasticizer were produced for food packaging applications. Results showed improvements in homogeneity and thermal stability, contrarily reduction in high hydrophilicity of starch and water vapour transmission rate due to PHBV microparticles incorporation. Overall improvement was observed by adding PHBV microparticles in developed films [169].

One of the most recent reports concentrated on developing a low-cost manufacturing strategy and improving the performance of PHBV through reacting blending with an elastomer. Thermoplastic polyurethane (TPU) was combined with hexamethylene disocyanate
(HMDI) as a reactive extrusion agent to create PHBV blends. The results demonstrated that the compatibilizer HMDI reduces the average particle size of TPU and improves adhesion, resulting in an improvement in the blend's toughness and long-term behaviour performance. TPU, on the other hand, hampered the complete biodegradation of PHBV under standard conditions. Despite the fact that the blends are not considered biodegradable by current standards, this work shows how to improve the properties of PHBV in order to turn it into a plastic that can be used in injected parts, overcoming one of its most critical limitations, brittleness [152]. To avoid direct food contact with biological and physical contaminants, another recent study prepared a thin flexible trilayer blend film with a core layer of thermoplastic starch (TPS) sandwiched between two PHBV layers (80/20). The study confirmed an increase in tensile strength, as well as excellent barrier properties and biodegradability, indicating the material's potential for use in sustainable food packaging applications [170].

**PHBV/nanocellulose**

Several studies have found that nanocellulose is suitable for potential applications like sustainable packaging [171,172], biomedical applications [173] and an excellent choice for PHBV reinforcement, some are mentioned in table 6. Based on their morphology, nanocellulose is divided into two types: cellulose nanocrystals (CNCs) (also known as CNW) and cellulose nanofibers (CNFs) [174,175]. CNCs outperform CNFs in PHBV nucleation, but CNFs are more thermally stable. At an equivalent nanocellulose concentration, CNCs produced PHBV composites with a higher tensile modulus than CNFs. However, with low content, both types of nanocellulose reinforcements perform better [176]. Cellulose nanocrystals improve the thermal stability, mechanical properties, melt-processing window, modulated crystallinity, and hydrophilic properties of the resulting PHBV nanocomposites [177]. One study found that PHBV/CNCs composites had lower crystallinity than pure PHBV [178]. The hydrogen bonding interactions between the cellulose nanocrystals and the PHBV matrix were attributed to the significant improvements in mechanical properties [179]. Alternatively, by combining PHBV with nanofibrillated cellulose (NFC) as reinforcement, totally biodegradable composite materials (PHBV/NFC) with an almost twofold increase in stress-strain modulus were produced [180]. The PHBV matrix, however, may become much more thermally degraded with a high NFC content.

**Table 4. Nanocellulose based PHBV bionanocomposite.**

<table>
<thead>
<tr>
<th>PHBV/nanocellulose</th>
<th>Samples Composition</th>
<th>Processing Method</th>
<th>Enhancement in properties</th>
<th>Applications</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV/CNC</td>
<td>CNC (2, 4 and 6 wt.%)</td>
<td>Melt mixing/extrusion</td>
<td>Enhanced crystallinity, Young's modulus, and barrier properties</td>
<td>Plastic packaging material</td>
<td>[181]</td>
</tr>
<tr>
<td>PHBV/CNC</td>
<td>CNC (2, 4 and 6 wt.%)</td>
<td>Solvent casting method</td>
<td>Enhanced oxygen and water vapour barrier properties</td>
<td>Plastic packaging material</td>
<td>[182]</td>
</tr>
<tr>
<td>(PHBV)/(CNC) and aluminum oxide nanoparticles (Al₂O₃)</td>
<td>CNC and Al₂O₃ in PHBV (0, 1, 3 and 5 wt.%)</td>
<td>Solvent casting method</td>
<td>Physical and mechanical properties are improved, crystallinity and glass transition temperature are increased, and flexibility is reduced.</td>
<td>Composite films</td>
<td>[15]</td>
</tr>
<tr>
<td>PHBV /CNCs.</td>
<td>CNC (1, 2, 3, 4, 5, and 6 wt.%)</td>
<td>Melt compounding</td>
<td>Thermal stability and mechanical properties have been improved</td>
<td>Composite material</td>
<td>[183]</td>
</tr>
<tr>
<td>PHBV/CNFs</td>
<td>CNF (1, 2, 3, 4, 5, 6 and 7 wt.%)</td>
<td>Melt compounding</td>
<td>Thermal stability and mechanical properties have been improved</td>
<td>Composite material</td>
<td>[183]</td>
</tr>
<tr>
<td>PHBV/CNF/ supermagnetic Fe₃O₅ NPs</td>
<td>CNC (1, 3, and 5 wt.%)</td>
<td>Solvent casting method</td>
<td>Magnetic nanocomposite films with enhanced mechanical and thermal properties, crystallization capability, and biocompatibility</td>
<td>Sustainable smart packaging materials</td>
<td>[14]</td>
</tr>
<tr>
<td>PHBV/bacterial cellulose whisker (BCW)</td>
<td>BCW (0.3, 0.6, 0.9, 1.2, 1.5 and 2 wt.%)</td>
<td>Solution blending</td>
<td>Mechanical properties are improved with piezoelectric properties.</td>
<td>Fully degradable green packaging film material, promote bone repair</td>
<td>[184]</td>
</tr>
<tr>
<td>PHBV/CNCs</td>
<td>CNC (1, 3, and 5 wt.%)</td>
<td>Solution casting method</td>
<td>PHBV’s thermal stability, crystallization rate, and crystallinity index improved.</td>
<td>Biocomposite films</td>
<td>[185]</td>
</tr>
<tr>
<td>PHBV/(CNFs) and lignocellulose nanofibrils (LCNFs)</td>
<td>NDR Electrospinning coating technique</td>
<td>Improved water contact resistance, more balanced mechanical properties, and higher barrier performance against water vapor</td>
<td>Food packaging applications</td>
<td></td>
<td>[186]</td>
</tr>
<tr>
<td>PHBV/NFC</td>
<td>NFC (0, 2.5, 5, and 10 wt.%)</td>
<td>Melt compounding</td>
<td>Tensile modulus, storage modulus, and desorption diffusivity were all increased, but toughness deceased.</td>
<td>Packaging</td>
<td>[180]</td>
</tr>
<tr>
<td>PHBV/CNC</td>
<td>CNC (0, 1, 3 and 5 wt.%)</td>
<td>Solvent casting method</td>
<td>Improved tensile strength and thermal stability, but mechanical properties for more than 3% by weight of the composites</td>
<td>Nanocomposite films</td>
<td>[187]</td>
</tr>
<tr>
<td>PHBV/cellulose nanocrystals (CNCs)/ (aqueous) poly(vinyl acetate) (PVAc)</td>
<td>CNC (2.4 and 4.8 wt.%)</td>
<td>Melt mixing/extrusion</td>
<td>Higher tensile strength and elongation at break</td>
<td>Biodegradable packaging</td>
<td>[188]</td>
</tr>
</tbody>
</table>

*NDR: no data reported, CNC: Cellulose nanocrystals, CNF: Cellulose nanofiber
The most studied polysaccharide nanocrystals are cellulose nanocrystals (CNCs) or Cellulose nanowhiskers (CNWs) and have also proven effective as a PHBV reinforcement. In last decade, several attempts have been reported in enhancing the mechanical and thermal properties of CNC based PHBV bionanocomposites. Studies reported mostly used solution casting technique for fabrication of composite films, which limits low efficiency and environmental hazard. Melt compounding techniques, on the other hand, are environmentally friendly (solvent-free) and industrially and economically feasible for processing thermoplastic polymers [183]. Further, to change the mechanical properties of composites, the ability to control fibre orientation is highly desirable. CNWs were successfully oriented in the PHBV matrix using an electric field, with the CNWs aligned in the direction of the applied electric field [189].

Similarly, Solution casting technique was used to prepare biodegradable films of PHBV/CNCs for packaging applications. Furthermore, PHBV/CNWs nanocomposites exhibited significant mechanical anisotropy and excellent barrier properties at low CNW concentrations (4wt.%) [182]. The same research team later reported the fabrication of PHBV bionanocomposites with CNCs via extrusion (melt mixing technique). Experimental results indicated better mechanical and barrier properties at low filler loading, confirming extrusion as potential alternative for packaging applications [181]. Similar behaviour was observed when, the inclusion of CNCs increased the tensile strength and thermal stability of PHBV/CNCs nanocomposite films, on the other hand, CNCs loading (3wt% and more) severely agglomerated in the biopolymer matrix, lowering mechanical characteristics [190]. Further, nanocomposites of PHBV with cellulose nanocrystals (CNCs) have recently been prepared and processed using various methods. Only in the case of peroxide-aided extrusion did tensile strength improve (by 13%) [191].

Fabrication of ternary nanocomposites is reported, Polyhydroxybutyrate (PHB) and poly-(hydroxybutyrate-co-hydroxyvalerate) (PHBV) masterbatches with cellulose nanocrystals (CNCs) were prepared by dispersing CNCs in (aqueous) poly(vinyl acetate) (PVAc) emulsion or poly(ethylene glycol) (PEG) solution, followed by melt mixing of dried PVAc/CNC and PEG/CNC masterbatches with neat, with CNC contents of 2.4 and 4.8 wt.%, intended for biodegradable packaging applications. Experimental results indicated that PVAc addition improved elastic modulus and tensile strength, and elongation at break is increased by increasing the CNC content. In addition, microscopic examination revealed a lesser amount of nanocrystal aggregates in the composites than was previously reported for binary nanocomposites made by mixing directly from melts, such as PHB/CNC and PHBV/CNC [188]. CNCs have a high tendency for agglomeration during melt processing, limiting their ability to improve mechanical properties [184]. There has been little research into melting techniques for the processing of nanocellulose/PHBV nanocomposites. Furthermore, acid hydrolysis of CNCs reduces nanocellulose’s thermal stability, limiting its operating temperature range [192]. In the view, recently, bacterial cellulose whisker (BCW) was acetylated to reduce the agglomeration effect of hydrogen bonds on its surface, improve thermal stability and compatibility with the PHBV matrix. Experiment results showed that ABCW has both a heterogeneous nucleation and a hindrance effect on the PHBV matrix. As ABCW content increases, PHBV gradually decreases from large spherulite and then increases, and crystallinity decreases. According to the study, the mechanical properties of the composite are best at 1.2% wt% of ABCW. The outcomes demonstrate that ABCW is a useful toughening filler for PHBV. On the one hand, PHBV/BCW composite can be utilized to produce completely biodegradable green packaging film. Furthermore, because of the hot-pressing processing used in this paper, no additional mixing occurs during the moulding process, and the result is ideal after internal mixing or extrusion [184].

**PHBV for sustainable food packaging**

PHBV has already piqued the scientific community’s interest due to its critical biocompatibility and biodegradability in a variety of environmental conditions. Because of its remarkable properties. The majority of the applications of PHBV focused on packaging, reinforced materials, and biomedical applications such as tissue engineering etc, shown in Fig. 7.

![Fig. 7. Industrial packaging and biomedical applications of PHBV reinforced materials used in various industries.](https://aml.iaamonline.org)

Packaging requirements need to be fulfilled depending on application from any material like material properties and permeability, proper wrapping and shielding, toxicity, preservation of food, maintenance under storage conditions, biodegradation of packaging and more [193, 194]. For packaging materials, PHBV reinforcement from plant fibres provides low cost production, good resistance, non-toxicity, high rigidity and specific modulus, and excellent biodegradability [195]. Recently, Opuntia ficus indica cladodes plant (with high cellulose content) reinforced PHBV biocomposites were prepared by melt compounding. The results showed improved tensile properties, low density, high rigidity, and thermal stability,
confirming their suitability for packaging materials and contributing to the improvement of the ecosystem [196]. Natural fillers, on the other hand, limit polymer chain mobility and thus biocomposites melt processing [197]. To improve this behaviour, additives are used, which increases toughness and processability. To prepare biocomposites from PHBV/(maleated polybutylene adipate-co-terephthalate)(mPBAT) reinforced hemp powder residue, melt compounding is used. Improved interfacial adhesion caused by maleated PBAT (mPBAT) via reactive extrusion processing could explain the improved mechanical and thermal properties over neat PHBV. Overall, the developed biocomposite could be a viable candidate for rigid packaging applications [197].

Food packaging

Functions

Containment

Protection or preservation

Convenience

Communication or Marketing

Moisture

Water

Grease

Mechanical protection (UV radiation, etc.)

Chemical protection (Oxygen, carbon dioxide, aromatic compounds, etc.)

Microbial protection (Microbial metabolites, etc.)

Fig. 8. Functions of food packaging depends on number of factors like safety, storage, shielding, transportation etc., adapted from Tan et. al. [200].

In the context of growing environmental concern, PHBV, a natural biopolymer produced by bacterial action, has emerged as a promising option for food packaging applications [202,203]. Furthermore, compatibilizers, coupling agents, and other surface modification strategies are used on packaging materials to improve their performance before they are used in multilayer packaging films or nanocomposites [17,204]. Because of the combination of properties of PHBV, it has sparked considerable interest as an eco-friendly biopolymer for packaging use [205].

The main concern with food products nowadays is protection against microbial contamination [206]. To increase the shelf life of food that has been preserved, innovative packaging materials that provide antibacterial action for a long period are needed [207]. According to one study, ternary composites with nanohybrids of silver and cellulose nanocrystals incorporated in PHBV via solution casting showed better mechanical, overall migration, barrier, and antibacterial properties, as well as better thermal stability. These findings with a 99.9% antibacterial activity imply that these composites have potential for use in food packaging [208].

Active packaging, another cutting-edge method for microbial growth control, enhancing the food quality and safety of packaged foods. Nanocomposites films were produced using PHBV and stabilized silver nanoparticles (0.04% wt). The results showed that nanocomposite films had 56% less oxygen permeability than pure PHBV, as well as prolonged (up to 7 months) antimicrobial activity against the most common food-borne pathogens, Salmonella enterica and Listeria monocytogenes [209]. However, in another report, the antibacterial effect of PHBV films with oregano or clove essential oil, or their main compounds,
carvacrol (CA) and eugenol (EU), respectively, were analyzed in food matrices (cheese, chicken breast and pumpkin and melon) and in vitro test for Escherichia coli and Listeria innocua. It was observed that there are several compositional factors that affect the active compound’s availability to exert its antibacterial action in a specific food. Therefore, antimicrobial analyses are required that are specific to the food in question to ensure the effectiveness of a particular antimicrobial packaging material [210].

Bacteria cells can adhere and grow in favourable conditions, forming a biofilm that can degrade food quality and safety. Pretreating the sensitive surface with an antimicrobial agent is one approach to microbial adhesion. Some materials can resist bacterial colonisation when impregnated with biocides or antibiotics. Moreover, several studies have reported the incorporation of inorganic nanoparticles in PHBV as reinforcing agents for improving mechanical and functional properties such as antimicrobial activity. In addition, ZnO nanoparticles are non-toxic and have been approved by the FDA as safe substances, and recent studies have shown that they do not cause DNA damage in human cells [211]. Several studies have already been published on nanocomposite films made from PHBV and ZnO nanoparticles via melt compounding for enhancing antimicrobial activities [212]. However, a small amount of inorganic antimicrobial agent causes agglomeration in the polymer matrix, while a large amount causes toxicity, further deteriorating the properties of food packaging [213,214].

Electrospinning is a highly effective method for producing nanofibers with improved physicochemical and functional properties without the use of chemicals or high temperatures, uses a wide range of polymer solutions, mixtures, nanoparticles, and antimicrobial agents that have a viscosity in solution suitable for producing nanofibers, piquing researchers’ interest in new food packaging systems [215]. Recently, the electrospinning process was used to coat PLA film with PHBV/ZnO (Fe-doped ZnO nanoparticles (1 wt%)) nanosystems. It was carried out at room temperature, with minimal energy consumption, and without the use of potentially toxic solvents. The PLA/PHBV/ZnO:Fex electrosprun nanosystems demonstrated excellent antibacterial activity against Pseudomonas aeruginosa (ATCC-27853) [216]. In another recent report, Salmonella Enteritidis bacteriophage Felix O1 was incorporated into polyvinyl alcohol (PVOH) coatings and fibres deposited by casting and electrospinning on polyhydroxybutyrate/polyhydroxyvalerate (PHBV) films for potential use as an anti-Salmonella agent. These findings demonstrated that both PHBV/coating films and PHBV/nanofiber films are suitable for food packaging to avoid bacterial contaminations, with added antimicrobial potential against Salmonella via Felix O1 bacteriophage encapsulation [205]. Bacteriophages are highly safe and effective antimicrobial agents with a long shelf life; however, bacteriophages have low stability in food environments, and electrospinning offers a significant advantage when incorporating bacteriophages that are temperature and solvent sensitive [217].

Furthermore, self-reinforcement could be an efficient approach, implying the production of biodegradable films using the same matrix and filler material. The solvent casting method was used for the first time to prepare biodegradable self-reinforced films (srPHBV) of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and PHBV microparticles. The incorporation of PHBV microparticles significantly improved the barrier properties against water vapour, oxygen, and carbon dioxide. The srPHBV films may be suitable for food packaging applications [218].

**Economic aspects for PHBV production**

Notably, developing PHAs bioplastics with biodegradability have made further inroads into the commercial industry, which is estimated to be valued USD 28 billion tons by 2025 [219]. In 2017, almost 350 million tons (Mt) of total plastics were manufactured, with biodegradable plastics accounting for only 0.6% of total output (2.1 Mt) [220]. PHAs currently occupy a market volume of 23 000 Mt, with a compound annual growth rate of 6.28% over the last four years [221]. The examination of PHA production costs is critical for the development of methods targeted at improving process performance. Typically, a techno-economic study takes into account fixed capital (both direct and indirect) and annual operating costs [222]. All of these expenses are heavily influenced by production conditions. Although PHAs are ecologically friendly and biocompatible polyesters, their costly selling price (4 to 5 euros/kg) in comparison with fossil-based plastics (less than 1 euro/kg) limits their commercial market rivalry or larger usage as commodity plastics [223,224]. According to current estimates, the costs of producing PHBV particularly range from 1.50 to 10 $/KgPHA, depending on the location of the production plant and, most importantly, the operational parameters used [66,225]. For instance, TianAn Biopolymer, a PHBV-producing firm with headquarters in China that uses the brand ENMAT™, has been published in the literature for its yearly cost and manufacturing capacity of 2000 Mt [226].

Reducing production costs has been a constraint for market expansion. To overcome such a difficulty, significant research efforts have focused on the increased exploitation of renewable and low-cost carbon sources for PHBV synthesis. Since lignocellulosic biomass is a plentiful, inexpensive, carbon-neutral, and non-edible raw material, the biotransformation of this biomass to PHAs has attracted a great deal of interest from both academic and industry sectors [227,228].

The utilization of natural fiber/fillers, particularly in the PHBV matrix, may be one approach to extending the commercialization of green composites. When compared to pure biopolymers, their utilization should result in improved mechanical qualities and lower manufacturing...
costs while maintaining full biodegradability [143]. In a work, solid winery by-products, specifically wine lees (WL), were employed as natural fillers within poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) to make biocomposites in various percentages (10, 20, and 40 phr). The results demonstrated how WL can improve the overall characteristics of biopolymers while maintaining their bio-based origin. The economic profits were calculated by comparing the final cost of composite to the cost of pure polymer. To broaden the economic analysis, profit surfaces at various WL contents have been created, with the initial polymer price ranging from 2.5 to 7.5 euros/kg and the process of extrusion cost ranging from 0.1 to 0.7 euros/kg [229].

The carbon source is also a substantial contributor to overall production costs. Raw materials account for 30-40% of total expenses, according to Chanprateep [230]. When the cost of the substrate was reduced, the cost of PHA production was reduced, and vice versa [26, 231]. Indeed, for most practical applications of PHBV, the 3HV fraction should be at least 10-20% (m/m), and expensive precursors (e.g. propionate and valerate) are frequently required [66].

The utilization of waste substrates, on the other hand, may necessitate additional expensive equipment expenditures due to pre-treatments [232]. Alternatively, when using specialized bacterial strains which require no precursors, it is possible to avoid sterilizing costs and/or ensure higher output. In terms of selecting the best substrate, both the primary and the waste substrates have been tested [49,50]. Kollet et al. conducted a techno-economic analysis of PHA production by comparing various microorganism species [233]. They calculated that P. hydrogenovora and H. mediterranei polymers could be synthesized at costs of 10.5 and 2.82 euros per KgPHA, respectively. Because of the increased PHBV concentration and production, H. Mediterranei had the lowest costs [34]. Its capacity to produce high-quality Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) from various waste feedstocks has been extensively researched [73,234].

The cost of feedstock is another major contributor, accounting for more than 40% of the overall yearly operating cost [235]. As a result, numerous academics are working on bioconversion technologies to use wastes or industrial byproducts which ran a sensitivity analysis, revealed more information: the production costs of PHA were greatly affected by variations in feedstock price [236]. Using waste as feedstock is a critical approach because it significantly reduces both substrate supply and waste disposal costs [106,110]. As a result, during the past few years, experts have examined a variety of waste materials to ensure their PHBV production efficiency. The usage of waste material, on the other hand, necessitates the employment of proper pre-treatment processes. The goal of pre-treatments is to speed up degradation and/or avoid inhibiting the activity of microorganisms [34]. Pre-treatments, on the contrary, incur additional costs. In the end, the economic analysis demonstrated that these biocomposites may be viable for large-scale applications, formulation based on economic and technological constraints.

CHALLENGES AND FUTURE PROSPECTS

The current challenge of producing PHBV biocomposites with balanced mechanical strength, thermal stability, antimicrobial activity with improved barrier properties, and processability can still be improved by employing diverse strategies described in this review. Furthermore, large-scale utilization of PHBV is still constrained by its high production costs and low productivity rate. Though biological approaches for producing PHBV from single strategies such as waste substrates from agro industries, food industries, waste water, and so on, as well as combined strategies, are discussed here, there is still much room for future research in the implementation of efficient and low-cost PHBV production processes. Finding new microbial strains capable of accumulating higher levels of PHBV, developing much more efficient fermentative routes, or lowering the costs of the polymer extraction process is also a major barrier to its widespread use. Innovative techniques such as high barrier double-side coatings, multi-layer packaging film techniques, and electrospinning have yet to be investigated.

To create advanced composites, PHBV can be modified by combining it with other polymers, natural fibres, carbon nanomaterials, nanocellulose, nanoclays, and so on. Despite extensive research in PHBV composite synthesis, there is still much room for the introduction of new biocomposites, particularly with biodegradable and biocompatible nanocelluloses, as there is very little research in this field. Additionally, active food packaging has been researched and used to extend food shelf life and monitor the conditions of packaging contents during transportation and storage. Incorporating effective adsorbing and releasing systems, such as oxygen scavengers, liquid and moisture absorbers, and antimicrobials, as well as time-temperature and freshness indicators, into biodegradable packaging will result in the next generation of biodegradable smart packaging. In other industries, PHBV packaging applications are very limited compared to food packaging, and studies on its functionalities with better composting properties are critical for future research.

As a result, more research on process scaling-up of PHBV is required in this research area. Nonetheless, the improved strategies presented here are capable of producing advanced biodegradable PHBV-based materials with improved properties, which are an excellent solution for many current advanced applications. Furthermore, these rapidly evolving enhancement approaches create new and exciting opportunities in this critical research field of eco-friendly and sustainable packaging.
AUTHORS BIOGRAPHY

Rahul Dev Bairawan completed his BE in Mechanical Engineering and M. Tech in Production Engineering from Govind Ballabh Pant Engineering College, Paugi Garhwal, Uttarakhand, India. Currently he is a PhD student at School of Industrial Technology, University Sains Malaysia. His research interests are natural fibres, biocomposite materials and biopolymers.

Esam Bashir Yahya attended Al-Mergib University and obtained his first degree in Microbiology in 2014 before going on to earn his master degree at Lincoln University College and PhD at the University Sains Malaysia. Currently, he is a lecturer and researcher in bioprocess division, School of Industrial Technology University Sains Malaysia. His research interest is biotechnology, drug delivery and tissue scaffolding. He has published more than 35 research articles in high impact journals.

Dr. Deepa A Gopakumar is an academic researcher currently working as a Prestigious Chief Ministers Nava Kerala Post-Doctoral Fellow at Cochin University of science and technology. His research of interest focuses on the varying technological applications of nanocellulose and its composites. He has an H-index of 22 co-authored more than 42 publications in more than 2000 citations into his credit.

Prof. Abdul Khalil, HPS is currently a Senior Professor and Dean at the School of Industrial Technology, Universiti Sains Malaysia. He is a fellow of Institute of Materials, Minerals and Mining (FIMMM), United Kingdom. He is a member of America Chemical Society and a member of Wood Science and Technology. He is awarded with Malaysia’s Rising Star Award (Thomson & Reuters) in 2015 and Malaysia’s research star award (Scopus, Elsevier, High impact factor) in (2018).

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GRAPHICAL ABSTRACT

The advantages and limitations of the environmentally friendly PHBV biopolymer, as well as its production enhancing strategies, are discussed. Further, mechanical and functional properties can be improved by selecting an appropriate method for preparation of PHBV biocomposites for sustainable food packaging applications by adding suitable reinforcements to PHBV.