Polyhydroxybutyrates: A Sustainable Alternative for Synthetic Polymers

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Over the past decade, petroleum-based plastics have emerged as a significant concern, disrupting normal human life cycles. The adverse impacts of synthetic plastics on living organisms include their accumulation in both marine and terrestrial habitats, lack of proper disposal methods, slow biodegradation rates, and absence of natural degradation processes. Consequently, researchers have been driven to develop eco-friendly polymers that pose minimal harm to the environment. Among the most prevalent alternatives to synthetic plastics are biopolymers, with Polyhydroxybutyrates standing out as a widely used example due to its properties suitable for replacing conventional plastics. Biopolymers offer solutions to the drawbacks of synthetic plastics. When biopolymers are released into the environment, they do not generate toxic chemicals that harm living organisms. These biopolymers are already in use in various industries. Through this review, we would understand the usage of these biopolymers in various industries.

Keywords: Biopolymers; Biodegradable; Eco-friendly; Polyhydroxybutyrates.

Plastic is a flexible product and has the ability to adapt to different functional activities easily. It is of low cost and easy to produce in large numbers. It has numerous applications in various industries such as food, agricultural, and pharmaceutical[1]. However, the usage has resulted in wide problems over the last decade; incineration and fabrication of plastics have made air, water, and soil pollution worse. Waste management and treatment of plastics have become a major problem. A study states that per year 31.9 million tons of plastics had been dumped in the environment[2]. Also, a survey explains that over 60 million metric tons of plastics were produced in 1980, followed by 187 million metric tons in 2000, in 2010 was 265 million, and in 2017, 348 million were produced[3]. These issues have led scientists and researchers to find an immediate alternative to synthetic plastics, which have less or no effect on the environment.

Polymer materials have dominated global industries for the past 50 years due to their adaptability, durability, and low cost, so that we cannot imagine a product without them. In contrast, many synthetic polymers are made from petroleum and coal as raw materials, making them incompatible with the environment since they cannot be recycled naturally. Since synthetic polymers have a negative impact on the

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environment, a solution could include combining different types and sources of biological materials called biopolymers, including starch, cellulose, chitin, chitosan, zein, and gelatin, which could be gradually replaced by synthetic polymers for these purposes. Biopolymers, including those obtained naturally and those synthesized, are much more eco-friendly, user-friendly, and cost-effective than petroleum-based polymers. Biopolymers have certain advantages over petroleum-based polymers based on life cycle assessments. Additionally, petroleum-based plastics are more environmentally damaging than biopolymers. It has been demonstrated that biopolymers have advantages over synthetic polymers in medical, tissue engineering, military, and environmental applications[4].

Biopolymers are composites of monomers that are derived from living things. Due to its distinct characteristics, it has drawn the attention of researchers. They are structures that resemble chains and might deteriorate in the environment, also, their significance is derived from how they interact with other polymers. They make excellent gas and vapour sensors since they are widely available and biocompatible. Biopolymers are divided into three categories: natural, synthetic, and microbial biopolymers, depending on where they are produced. Biopolymers are molecules made up of carbohydrates, amino acids, and hydroxy fatty acids, which are linked together by enzymes to produce high molecular weight molecules. Biopolymers such as polysaccharides, polyesters, polyamides, and polyphosphates can be synthesized by bacteria. Polysaccharides such as cellulose and starch are also biopolymers produced by microorganisms incuding bacteria and fungi. Usually, polyhydroxybutyrates (PHB) are produced by microorganisms under unbalanced or nutrient-limited conditions. Other than acting as storage molecules, PHB also has other functions such as increasing the bacteria's resistance against the abiotic stresses and also serves as a protection against hydroxyl-radicals[5]. Despite these many advantages, there are some drawbacks in large scale production and usage of biopolymers in industries. It is because of the high cost of production that is considered as a major drawback for the production of biopolymers in large scale. Another drawback is that PHB alone cannot be used to produce a product. It must be blended with other ecofriendly materials to make the product more stable and to extend its lifetime. This is why the cost of production of the biopolymer is high. The researchers are very keen to overcome this drawback and replace all polypropylene or petroleum-based plastics with bioplastics or biopolymers. To retain the available resources and to avoid further damage to the environment, these alternatives must be developed as soon as possible.

Polyhydroxyalkanoates

PHAs, a type of polyester that is commonly used as a compound for the production of biopolymers, are well known as PHBs. PHBs, Polyhydroxyvalerates (PHVs), poly-4-hydroxybutyrates (P4HBs), Polyhydroxyhexanoates (PHHs), and polyhydroxyoctanoates (PHOs) are a few examples of polyhydroxyalkanoates which are biodegradable and environmentally non-toxic. It has a variety of qualities that can be altered according to the requirement.

PHAs produced by bacteria are classified based on their structural characteristics into three groups: short chain length (scl) PHAs (3-5 carbon atoms), medium chain length (mcl) PHAs (6-14 carbon atoms), and long chain length (lcl) PHAs[6]. The quantity of production is higher in scl-PHAs than mcl-PHAs[7]. The cost of PHA in the market is currently €5 per kilogram. It is 6 times more expensive than normal plastics which are already in use[8]. The carbon sources such as olive oil, fermented molasses, date syrup, pomace, and effluents from paper mills and palm oil mills have accumulated biodegradable PHAs of around 40 and 70%[9].

Polyhydroxybutyrates

There have been various microorganisms that have been found to produce PHBs, a copolymer of poly (3- hydroxybutyrate-co-hydroxy-valerate) utilizing biomasses consisting of lignocellulosic molecules[10]. PHBs produce granules that are not soluble in water and act as a storage unit of energy under stress conditions. Both Gram-positive and Gram-negative bacteria synthesize these PHBs intracellularly only when there is an excess of carbon and a lesser amount of other nutrients[11].

In 1925, the first PHB (polyester poly-3-hydroxybutyrate) was discovered by Lemoigne. It has a linear chain structure in both crystalline and amorphous phases, with high crystallinity and availability in both pure polymer and also as copolymers. It is mainly produced as a carbon storage unit under stress in most bacterial strains[12]. A sufficient amount of carbon supply (simple sugars such as glucose, fructose, mannose, galactose, sucrose, and xylose or polysaccharides such as starch), lipids (oleates and glycerides), and nitrogen is the key component for the growth of microorganisms that produce PHBs. Nitrogen provides proteins, nucleic acid, and co-enzymes like vitamins for the growth of these microorganisms[13]. In 1983, researchers found that *Pseudomonas oleovorans* produce poly-beta-hydroxyoctanoate granules when grown on octane. That was the first time a PHA other than PHBs was identified[14].

PHB is a member of the PHA family, which is distinguished by an ester linkage group (-COOR) and methyl functional group (-CH_3). Functional groups present in the PHBs are the main reason for the material's hydrophobic nature, high crystallinity, thermoplastic, and brittle properties. Melting temperature (T_m) and glass transition temperature (T_g) are two temperatures that explain the thermal properties of the material[15].

Many bacterial species that produce PHBs have been identified and used. *Bacillus cereus*[16], *Cupriavidus necator*[17], *Pseudomonas aeruginosa*[18], *Azospirillum ruburum*[19], *Brevundimonas* spp., and *Enterococcus* spp are few bacterial species isolated from the wastes of the cardboard industry with the ability to produce PHBs[20]. Few other species, such as *Burkholderia cepacian*[21] *and Pseudomonas putida*[22] isolated from the biodiesel-glycerol and vegetable oil wastes, respectively, also have the ability to produce PHBs. Recent reports also explain that *Bacillus megaterium, Bacillus siamensis, Bacillus subtilis, Staphylococcus aureus*[23], *Paraburkholderia* spp.[24], *Methulocystis* spp.*, Rhizobium* spp.[25;26], *Aeromonas hydrophia, Burkholderia sacchari, Acinetobacter* spp.*, Halomonas boliviensis, Sphingobacterium* spp.*, Caulobacter* spp.*, Brochothrix* spp.*, Ralstonia* spp., and *Yokenella* spp.[27] also have the potential to produce PHB. Recently, it was reported that eukaryotic algae such as *Chlorella*[28] and *Botryococcus*[29] have the ability to produce PHB naturally.

Mechanism of PHB production and regulation

Microorganisms produce PHBs through various mechanisms. The production mechanism mainly depends on whether the chief source is structurally similar or not similar to the PHB structure. For example, hydroxyalkanoic acids are structurally related, while glucose is structurally unrelated to the PHB structure.

The most common pathway observed in most microorganisms is the formation of PHB from two acetyl-CoA molecules. There are three main phases involved in this biochemical mechanism through which PHBs are produced. The first phase involves the formation of acetoacetyl-coenzyme A. Utilizing 3-ketothiolase (PhaA), two molecules of acetyl-CoA are condensed to form acetoacetyl-coenzyme A (CoA). PhaA enzyme is an acetyl-CoA acetyltransferase, also known as acetoacetyl-CoA thiolase, which assists in the condensation of two units of acetyl-CoA to acetoacetyl-CoA. The second phase involves the formation of 3-hydroxybutyryl-CoA. In this step, acetoacetyl-CoA is reduced by nicotinamide adenine dinucleotide (PhaB) to generate 3-hydroxybutyryl-CoA. Here, one molecule of NADPH is oxidized to $NADP^+$. The third phase involves the formation of PHB by utilizing the PHB synthase (PhaC). 3-hydroxybutyryl-CoA formed in the previous step is polymerized, releasing CoA to form PHB[30]. Microorganisms such as *Aeromonas hydrophila* produce PHB using both acetyl-CoA and beta-oxidation pathways simultaneously[31].

PhaR/PhaP gene is the main regulatory gene involved in the regulation of PHB production. To observe the regulatory conditions, the phaR gene was mutated and compared to the PhaP1 and PhaP4 double mutated genes. The results showed that under impaired conditions, PhaR gene showed less PHB production when compared to PhaP1 and PhaP4 double mutated genes. Other than that, PhaR negatively regulated the PhaP1, PhaP2, and other genes such as PhaA1, PhaA2, PhaC1, and PhaC2 genes involved in the PHB production. By these, the researchers understood that PhaR gene, by controlling the expression of phasins and biosynthetic enzymes, regulates the PHB granule formation[32].

Proteins associated with PHBs:

PHBs have spherically shaped, highly organized structures known as PHB granules or carbonosomes. The proteins related to PHB granules are named granule-associated proteins

(GAP), located on their surface. It has many roles, such as structural, biosynthetic, catabolic, and regulatory functions. Other than GAP, low-molecular weight proteins named phasins are also attached to the surface of PHB granules[33]. Unlike GAP, phasins are rare proteins that avoid interaction with other proteins and shield the hydrophobic PHB surface from hydrophilic cytoplasm. An example of phasin is PhaP1, which is present in *Cupriavidus necator*[34].

GAP also has other functions than covering the granule surface. PhaM, a granule associated protein present in *C. necator,* binds to PhaC chromosomal DNA and plays a role in the equal distribution between the daughter cells. PhaM influences the PHB production by activating the PHB synthase (PhaC1)[35].

Application of PHB in various industries

PHB is the most widely and popularly used alternative for non-biodegradable plastics such as polypropylene. PHB has properties such as tensile strength, tensile modulus, and melting temperature, similar to polypropylene. However, PHBs are biodegradable, while polypropylene doesn't have that property. Unlike polypropylene, they are biocompatible and doesn't release any toxic substances[36]. They are more suitable to replace polypropylene in many industries, but a few limitations make them difficult to use. The limitations include high-cost production, low thermal stability, high degree of crystallinity, brittleness, and hydrophobicity[37;38].

The PHB can be biodegraded in soil, water, and both aerobic and anaerobic environments. It can be degraded by the microorganisms containing extracellular depolymerases. The aerobic degradation of PHB will lead to the release of CO_2 and H_2O. In anaerobic degradation, in addition to CO_2 and H_2O, methane is also released into the environment. The degradation activity depends on different parameters such as pH, temperature, moisture, microbial activity, and PHB molecular weight[39].

PHB are combined with other materials to overcome their limitations. Some examples include hyaluronic acid (HA), polycaprolactone (PCL), polylactic acid (PLA), polyethylene glycol (PEG), chitosan, and other material[38]. PHB finds its application in various biomedical, pharmacology, packaging, and agricultural industries.

PHB in medicine and drug delivery

The inflammation caused by macrophages exposes PHB to extracellular liquids and cells, resulting in the degradation of the polymer into monomers and oligomers of 3-hydroxybutyrate[40]. This degradation property has made PHB a good candidate for the delivery of drugs[41]. The PHB was incorporated with an inhibiting agent called ursolic acid against tumor proliferation, producing antitumor PHB nanoparticles. This is done to increase the activity, availability, and delivery of the ursolic acid in PHB nanoparticles against HeLa cells. This study also reveals that the ursolic acid release is more efficient at 96hr, and the number of dead cells was high at that time[42].

Parsian[43] had designed PHB coated magnetic nanoparticles, which are loaded with gemcitabine (GEM-PHB-MNPs) to treat breast cancer. Also observed that the gemcitabine is released from the PHB coated nanoparticles only during an acidic environment i.e., during the presence of tumor cells, the nanoparticles are not cytotoxic to normal cells.

Extended-spectrum antibiotics loaded in PHB microspheres and PHB nanospheres are used to prevent infections caused by surgeries. Antibiotics such as sulbactam ampicillin or cefoperazone and gentamicin are loaded in the PHB for drug delivery[44].

Natural and synthetic polymers such as PHB are investigated by researchers for the production of fibrous materials[45]. The researcher successfully blended collagen with PHB to form fibrous scaffolds using TFA co-solvent, which can be used in cartilage engineering. They also found that the material produced had high hydrophilicity and a high weight loss rate with suitable mechanical properties[46].

Zhou[47], by electrospinning technique, constructed a biocomposite using chitosan and PHB. Through this research, it was found that with different percentages of PHB and chitosan, medical devices can be constructed with controlled degradation rate.

The use of biopolymers can also lead to promising medical applications such as implant materials which are immune to human immune responses. [48]. It had become an ideal material for the delivery of antimicrobial compounds to a target site and nano-entrapment[49]. PHB also provides an antimicrobial effect to titanium loaded with antibiotic implants, which is used to prevent infections caused by synthetic implants[50].

As PHBs are well tolerated by the immune system, they can be widely used to create surgical mesh, medical devices, orthopedic pins, surgical sutures, stents, repair patches, heart valves, staples, and screws[51]. Microcapsules made of PHB can be utilized to enclose Langerhans cells to restore insulin production and release; as a result, it can also be used as packing material for tablets[52;53].

PHB in tissue engineering

An extracellular matrix of rabbit chondrocytes was grown on polyhydroxybutyrate-co-hydroxyhexanoate (PHB-co-PHH) scaffolds[54]. Researchers also observed an increased production of collagen in PHB-co-PHH than in normal PHB. For wound dressing and ocular implants, PHB based composites were used, as well as scaffolds for bone implants[55]. Other than these, artificial tissues of retinal, tendon, bone, cartilage, and muscle have been developed using PHB-based composites[56;57].

PHB and piezoelectric materials in bone tissue regeneration

The use of polyhydroxybutyrate-co-valerate (PHBV) based piezoelectric material has shown a great result in bone tissue engineering. It has amazing biocompatibility and has the ability to make the material attractive in some tissue engineering related applications, such as the construction of functional scaffolds[58].

The researchers had developed PHBV or chitosan nanocomposite scaffolds embraced with Nano-Hydroxyapatite (nHA), which had been proven to be an alternative for bone tissue engineering due to its osteoconductivity and biocompatibility[59]. These scaffolds are further fabricated using 3D printing technology, which has been used to improve osteogenic differentiation and cell proliferation for bone tissue regeneration[60]. To improve bone tissue regeneration and osteoblast proliferation, bioactive glass nanoparticles embraced with PHBV scaffolds have been used[61].

PHB in food packaging

PHB acts as a barrier against water vapour, oxygen, and carbon dioxide and is stable, flexible, and highly resistant, which makes them suitable for use in food packaging[62]. They are also biodegradable and non-toxic to the environment, unlike polypropylene, widely used in bottles and jars manufacturing[63]. Coconut Fibers blended PHB composites have exhibited better thermal stability and good tensile properties, which has made them better for use as a plastic bag that can be recovered as seeds and planted[64].

PHB in other industries

Biomaterials can be produced by fabricating biopolymers, which can be used for many new industrial based application purposes[65]. As environmental pollution has become an increasingly serious issue in today's world, biopolymers have largely been used to tackle various challenges that have yet to be resolved[66]. To improve the functional and physical properties of biopolymers, materials such as essential oils, nanomaterials, bioactive components, and nanomaterials are used[67]. Few biopolymer-based biomaterials are microfibers and nanofibers, which are used in textile industries[68].

Due to its biodegradable properties, PHB can be used for foils and films production. It has become a flawless material for packing materials such as diapers, sanitary towels, shampoo bottles, disposable hygiene products, milk cartons, and razors due to its water-resistant property[69].

In recent years, PHBs have replaced commercial plastics to produce eco-friendly grow bags, protection nets, and compostable greenhouse films[70]. Based on the PHA copolymer of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), biodegradable mulch has been produced and patented by Danimer Scientific[71;72].

To improve crop growth and for the protection of crops from insects, birds, and natural climate fluctuations, biodegradable PHBs based agriculture nets are produced and used. It also helps the crop from overheating. Unlike normal plastics, these are biodegradable plastics that can be directly disposed of in the soil[73]. It also works as a microbial growth matrix that is friendly to roots and helps in water denitrification[74].

PHB in synthetic biology

PHB production in the microorganisms can be enhanced by using some of the synthetic biology and genome editing approaches. Ribosome-binding (RBS) optimization, cell morphology engineering, promoter engineering, chromosomal integration, downstream processing, and cell growth behaviour reprogramming are some synthetic biology approaches used to increase PHB production. The most recent approach used to increase the PHB synthetic pathways is CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) or CRISPR-associated protein 9 (Cas9)[75].

RBS and promotor engineering of genes in phbCAB operon, which is responsible for the PHB accumulation, has been proven to improve the transcription levels that directly influence the PHB production in the microorganisms[76]. RBS is of high accuracy and can be used to control gene expression using the calculator designed specifically for this method[77]. Using this method, a clone of phbCAB operon has been produced from *Cupriavidus necator,* which was used to optimize PHB production pathway in *Escherichia coli*. The cell dry weight has been in the range of 0% to 92% in the *E. coli,* which was genetically engineered by the RBS method, indicating the efficiency of this method[78].

Other than the RBS method, the promoter engineering method can also be used to regulate gene expression. It is one of the most common and powerful tools used to promote gene expression in the microorganisms next to the RBS method. Inducible hybrid promoters such as P_{lacl} and P_{trp} were developed using this method[79]. These inducible promoters were successful in promoting gene expression in *E. coli;* however, they were not able to promote gene expression in some organisms, such as *Halomonas bluephagenesis*[80]. By this method, the researchers were able to synthesize P3HB4HB, which has resulted in the >100 g/l cell weight with the productivity of 1.59 g/(l^{-h}) that contains 80% poly(3-hydroxybutyrate-co-4-hydroxybutyrate)[81].

CRISPR/Cas9 Method for phb synthesis

CRISPR/Cas9 is the most widely used technique for genomic editing in recent times. It is not only used for genomic editing but also for gene deletion, insertion, and also for the replacement of genes in some eukaryotic and bacterial cells[80]. It has been extensively used in both model and non-model organisms such as *E. Coli, Bacilli subtilis*, *Clostridium beijerinckii, Corynebacterium glutamicum, Lactocoocus lactis, Streptomyces spp., Cupriavidus necator, Klebsiella pnemoniae,* and *Halomonas bluephagenesis*[82;83;84;85;86;87]. A model organism *E. Coli* has been modified using CRISPR/Cas9. The genes such as *pflb*, *IdhaA*, *adhE*, and *fnr*, which are related to the by-product formation, have been deleted. With the pntAB overexpression, which catalyzes the conversion of NADH and NADPH increased, the PHB production resulted in cell growth[88]. *Cupriavidus necator* and *H. bluephagenesis,* which are non-model organisms, were used by the researchers in *Cupriavidus necator.* They were able to edit five genes with an efficiency range of 78% to 100% using inducible pBAD promotor[89]. In *H. bluephagenesis,* they were able to reach an efficiency of 100% using CRISPR/Cas9. Deleting the prpC gene from *H. bluephagenesis* using CRISPR/Cas9 resulted in the production of PHB in *H. bluephagenesis*[90]. Recently, it has created more attention and also led to the development of inclusion bodies. Researchers had changed the cell morphology from rod to sphere shaped by eliminating mreB, an actin-like protein gene[91]. The other proteins that are involved in the cell division, such as FtsZ, SulA, and Ftsz inhibitor MinCD, have also been engineered to manipulate and control PHB production[92].

Genome editing techniques such as RBS, promotor engineering method, and CRISPR/Cas9 play a vital role in the synthetic PHB production pathways and their regulation. These techniques help microbes to produce PHB quickly and also increase production when compared to normal microbes. Other than these techniques, more methods can be developed which will be useful for the PHB production industries[89].

A major drawback of the use of PHB in developing a product is its cost. The cost of microbial PHB production depends on both upstream and downstream processes. To reduce the cost of upstream processes, many techniques, such as the usage of low-cost carbon sources, economically synthetic pathway development, and many ingenious techniques for concentration, purification, and formulation of the products, were developed[93]. For downstream processing, some changes in the behaviour of cell growth and shapes have been developed using synthetic and genome editing techniques.

Increased PHB production and easy downstream recovery of product through sedimentation or filtration can be done when the FtsZ gene is inhibited. The inhibition of this gene leads to abnormal cell growth and filamentous cell formation, which will directly lead to more intracellular space for product accumulation[94]. Another example is the change in cell division patterns in *E. coli* caused by disrupting the MinC and MinD cell regulators. By disrupting the cell regulators, the binary fission is changed into multiple fission, which leads to a higher accumulation of PHB[95].

Advantages and drawbacks of biopolymers

Biopolymers are widely used instead of synthetic plastics due to its eco-friendly and easily degradable nature. It has become an alternative to petroleum-based plastics in a short period of time. The researchers have also identified that these biopolymers have the potential to reduce global warming. The rate of recycling of biopolymers is high, and it releases fewer toxins into the environment when compared to synthetic plastics. Due to its biocompatible and biodegradable nature, it is widely used in many industries, such as food and agriculture. These biopolymer-based products are widely used in the medical field as implants, material for drug delivery, scaffolds, and dressings. The greater advantage of these microbial biopolymers is that it can be easily manipulated according to the needs of the industries.

In industrial scale, commercial PHA production requires expensive raw materials and chemicals as sources of organic matter. The costs associated with fossil-fuel plastic production must be offset in order for this technology to be economically viable[96]. At lab-scale, many operating alternatives have been proposed to increase profitability and make the system easier to implement in the plastics market. Using industrial by-products and waste streams to create PHA has the advantage of being a more environmentally friendly method. Examples include agriculture feedstock, waste plant oils, and wastewater[97,98].

However, there are a few drawbacks to completely replacing synthetic plastics with biopolymers. Due to the biopolymers limited mechanical properties, high-cost production, and low processing capabilities, they are not widely used in industries as an alternative to synthetic plastics. The PHBs must be blended with other materials to make it flexible to use, which increases their cost of production. Due to high production, many industries are unable to completely replace synthetic plastics with biopolymers and researchers are working and trying to figure out methods to reduce productivity costs.

CONCLUSION

Synthetic plastics were most widely used and disposed of in the environment without proper treatment. These disposed wastes remain in the environment for longer periods of time, and accumulate in the soil for a prolonged time. This accumulation has led to many environmental concerns, causing harm to human life. The only way is either to identify how to degrade these synthetic plastics or to identify an alternative for the synthetic plastics. Researchers are also keen to identify an alternative. As a result, biopolymers were identified, which can be produced from different biological sources. The production of polyhydroxyalkonates (PHAs), such as polyhydroxybutyrates (PHBs) from microbes, represents a promising and sustainable avenue in this field. This has several advantages, which include environmental friendliness and renewable resource utilization, and it also aids in reducing dependence on fossil fuels.

As a biodegradable alternative to traditional petroleum-based plastics, PHB holds immense potential to address the escalating environmental concerns associated with plastic waste. Industries and consumers increasingly prioritize sustainable practices, and the production of biopolymers from microbes offers a viable solution to reduce the ecological footprint of plastic materials. Collaborative efforts between academia, industry, and policymakers will play a pivotal role in accelerating the adoption of microbial-based biopolymer production, fostering a more sustainable and circular economy. In the quest for eco-friendly alternatives, paving the way for a greener and more sustainable future.

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Conflict of interest

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Authors' contribution

All authors contributed to the conception and design of this study. Material preparation, data collection and analysis were performed by Balakumaran MD and Swetha J. Manuscript was proof read and approved by Balakumaran MD, Uma A, Ananth C, Nithya K, Swetha J.

Data Availability

All data and materials are available with corresponding authors.

Ethics Approval

Not Applicable.

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