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REVIEW ARTICLES

Applications of Polyhydroxyalkanoates in Drug Delivery

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SUMMARY

Polymers are of tremendous importance in the delivery of drugs to the site of action. Natural polymers are preferable to synthetic ones because they are biocompatible and biodegradable. Polyhydroxyalkanoates (PHAs) are polyesters of various hydroxyalkanoate monomers accumulated by a wide range of bacterial strains in the form of intracellular granules, under unbalanced growth and excess carbon conditions. Polyhydroxyalkanoates have been tipped as a potential replacement for synthetic polymers due to their superior biocompatibility, biodegradability and controllable retarding properties. However, the cost of production of PHA compared to the synthetic polymers they are envisioned to replace is high. Studies have been done to reduce cost by employing cheap and renewable carbon sources. This review article presents an overview of PHA production from these sources as well as the use of PHA in the delivery of drugs

Key Words: *Biopolymers, drug delivery, polyhydroxyalkanoate production, renewable polymers, polyhydroxyalkanoate*

Polihidroksialkanoatların İlaç Dağılımında Uygulamaları

ÖZ

Polimerler, ilaçların etki yerlerine ulaştırılmasında çok önemlidir. Doğal polimerler, biyouyumlu ve biyolojik olarak parçalanabilir olmaları nedeniyle sentetik olanlara tercih edilir . Polihidroksialkanoatlar (PHA'lar), çok çeşitli bakteri suşları tarafından dengesiz büyüme ve aşırı karbon şartları altında hücre içi granül formunda akümüle edilen çeşitli hidroksialkananoat monomerlerin poliesterleridir. Polihidroksialkanoatlar, üstün biyouyumluluk, biyobozunurluk ve kontrol edilebilir geciktirici özellikleri nedeniyle sentetik polimerlerin potansiyel olarak yerine geçebilir. Ancak, PHA üretim maliyeti, sentetik polimerlerle değiştirilmeleri ile karşılaştırıldığında yüksek olduğu öngörülmektedir. Ucuz ve yenilenebilir karbon kaynakları kullanarak maliyeti düşürmek için çalışamalar yapılmıştır. Bu derleme makalesinde kaynaklardan PHA üretimi yanında PHA'nın ilaç dağılımında kullanımına genel bir bakış sunulmaktadır.

Anahtar kelimeler: *Biyopolimer, ilaç dağılımı, polihidroksialkanoat üretimi, yenilenebilir polimerler, polihidroksialkanoat*

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INTRODUCTION

Polymers are a major part of the drug delivery system. They are essential for the provision of weight consistency as well as volume necessary for appropriate drug administration. Polymers are of importance in providing drug stability, release and targeting, improved bioavailability as well as patient acceptability (Beneke et al., 2009).

Synthetic plastics are useful in almost all facet of manufacturing, including pharmaceuticals. Their extensive usefulness can be attributed to the susceptibility of their structure to chemical manipulation. They are however mostly resistant to microbial degradation which makes their disposal problematic (Fletcher, 1993).

Biodegradable polymers, mainly of natural origins, are being gradually tipped to replace the non-degradable plastics. Biopolymers, being biocompatible, are being processed for various applications such as in the packaging industry, agriculture, chemical industry and medicine. Due to these, it is important to ensure large-scale production and extensive use of these biodegradable polymers. This will provide alternative sources of plastics and also benefit the environment (Zinn et al., 2001).

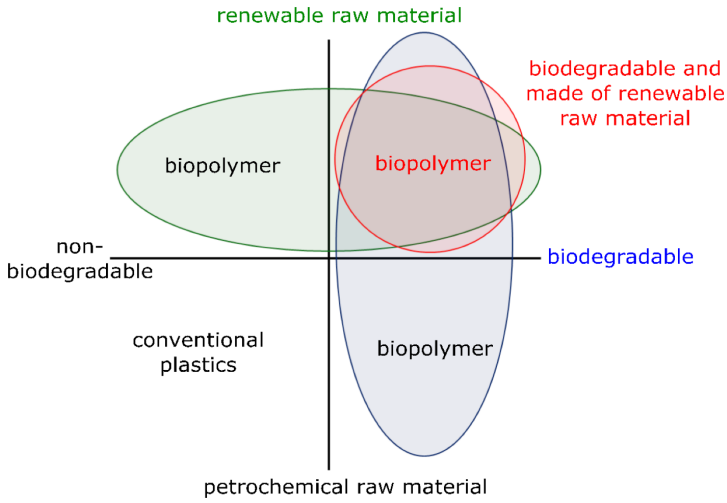

Figure 1. A Definition for biopolymers, including the stringent definition on the upper right (Reproduced from Pittmann and Steinmetz (2017) under the creative commons attribution - noncommercial (CC BY 4.0) license MDPI)

Microbial polymers are now being considered because of their biocompatibility and non-toxic properties. PHAs are polyesters of various hydroxyalkanoate monomers accumulated by a wide range of Gram positive and Gram-negative bacteria as intracellular granules, under unbalanced growth and excess carbon conditions (Verlinda et al., 2007). Poly(3-hydroxybutyrate) (PHB) is the first polymer in the PHA family. It is sometimes abbreviated as P(3HB) and has a hanging methyl group. PHB is produced by bacteria and algae, with a high degree of crystallinity (Moore and Saunders, 2013). It is brittle and rigid making it unsuitable for many applications. While PHB is biodegradable, its mechanical and physical properties are comparable to that of polypropene.

The advantages offered by poly(3-hydroxybutyrate) (PHB) and co-polymers, notably poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), compared to other synthesized polymers including polylactate, polyglycolate, and poly(lactide-co-glycolide) have shifted attention to their use in drug delivery as carriers or in tissue engineering as scaffolds. These advantages include biodegradability, excellent biocompatibility and easier processability. The retarding properties can also be controlled by varying the production process of the polymer and also the molecular weight of the constituent. An enhanced scope in the biomedical application is envisaged with a combination of PHB and other biocompatible and nontoxic polymers (Errico et al., 2009).

High cost has been an impediment to the production of PHA on a large scale. There is, therefore, the need for identifying and utilizing cheap and sustainable carbon source for industrial production (Sandhya et al., 2013; Urtuvia et al., 2014).

Production of PHA from cheap and renewable substrates

Poly-3-(hydroxybutyrate-co-hydroxyvalerate) was produced from olive mill wastewater (OMW) by extremely halophile *Haloferax mediterranei* in a one-stage cultivation step (Alsafadi and Al-Mashaqbeh, 2017). Media containing different concentrations of OMW were prepared and the optimum cultivation conditions were achieved in a medium that contained 15% OMW. A polymer yield of 0.2g/L and a PHA content of 43% PHA/cell dry mass were the highest obtained. These were obtained at 37^{0}C, 22% salt concentration and 170rpm. Analysis showed the biosynthesized PHA to be copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) of 6.5mol% 3-hydroxyvalerate. This copolymer was produced devoid of a fermentation step or the addition of an external substrate. The copolymer, compared to PHB, also showed lowered melting points at 140.1^{0}C and 154.4^{0}C.

Olive mill wastewater was also utilized to ascertain the possibility of PHA production from a pure culture of *Cypriavidus necator* (Martinez et al., 2015). An effluent rich in volatile fatty acid was produced by initially dephenolizing and fermenting the OMW. Different dilutions of the effluent (25%, 50%, 75% and 100%) were prepared and pre-grown cells of *C. necator* fed with the dilutions. Inhibition was observed with 75% and 100%. Further analysis indicated that the inhibition observed was majorly responsible for by the polyphenols. Two consecutive accumulation batch processes were carried out using 25% effluent, with no external carbon source. Through the process, up to 55% of cell dry weight of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) containing 11% hydroxyvalerate was accumulated.

Aremu et al. (2010) showed that cheap renewable hydrolysate of cassava starch reducing sugar can be utilized as a carbon source by *Pseudomonas aeruginosa* for polyhydroxy butyrate (PHB) production in batch fermentation processes. Extraction of starch was carried out from fresh cassava tubers and this was subjected to hydrolysis by the enzyme-enzyme method. The mixture of the hydrolysate and nutrient media in batch cultures was used as a medium for aerobic fermentation of *P. aeruginosa*. Gas chromatography method was then used to measure the PHB content. The result showed that PHB more than 50% of the cell dry weight was accumulated by the organism.

H. mediterranei has shown the ability to accumulate PHA on a high salt concentration medium. The product of an enzymatic reactive extrusion treatment of native corn starch was used as carbon source for growth of cell and PHA production (Chen et al., 2006).

Waste glycerol, a biodiesel industry byproduct, has been used as the carbon source for cell growth and PHB production in *C. necator* DSM 545 (Cavalheiro et al., 2009).

High yield of PHB was produced by *Ralstonia eutropha* NCIMB 11599 when saccharified waste potato starch was utilized as carbon source with phosphate limitation. Biomass 179g/l, 94g/l PHB and PHB productivity of 1.47g/l/h were achieved. While there was an accumulation of residual maltose in the fed-batch reactor, insignificant inhibition was however observed (Haas et al., 2008).

Beet molasses was used as a substrate for the synthesis of PHB (Yilmaz and Beyatli, 2005). It was found out that *B. cereus* M5 strain produced the highest dry cell mass (0.44g/l) in 4% molasses concentration. Higher PHB (73.84% PHB on dry cell mass) was produced by the strain in 1% molasses concentration, indicating that molasses concentration is inversely proportional to the strain's PHB productivity.

Ataei et al. (2008) produced PHA using 5% date syrup added with mineral salt medium as substrate. The PHA-producing bacteria were isolated from a date factory syrup waste. The colonies containing intracellular granules (identified with Sudan Black stain), grown in the syrup was incubated at 30^{0}C and shaken at 140 rpm for 60 hours. One of the strains yielded a PHB representing 71% cell dry weight. Another strain produced PHBV in date syrup media at a peak concentration of 2.2g/L and 10% valerate content in shake flask cultivation.

Crude glycerol was also used as a substrate for PHA synthesis by a mixed microbial community (Moita et al., 2014). A PHB yield of 47% cell dry weight produced at 0.27g/L by a mixed culture of aerobic strains was obtained. The final cost of the polymer is reduced by this process because the crude glycerol used in PHA production required no pretreatment step.

Poly-3-hydroxybutyrate-co-valerate (PHBV) was successfully synthesized by *R. eutropha* via oxygen limitation, using trade and industry and fruit and vegetable wastes, as the only source of carbon (Ganzeveld et al., 1999). The PHBV yielded from the concentration of the fatty acids in the organic waste was found to be 0.16g polymer/volatile organic matter.

Poly (β-hydroxybutyrate) has also been shown to be produced by *P. fluorescence*, strain A2a5. High PHB granules were accumulated in the sugarcane li-

quor medium cultured cells (Jiang et al., 2008). Sample from the cell, after purification, was identified to be PHB by gas chromatography and nuclear magnetic resonance analysis of polyester. PHB concentration of 31g/L was reached in shaking bottles. In 5L bioreactor, the yield of PHB obtained was 22g/L, which was up to 70% of the cell dry weight.

PHA can be produced by activated sludge with anaerobic feeding and discharge (AFD) process, using alkaline fermentation liquid of waste activated sludge (WAS) as a carbon source (Jiang et al., 2009). The report showed that this AFD process had the highest PHA yield compared to other reported processes of PHA synthesis. Subjecting activated sludge to the AFD process resulted in a PHA yield of 72.9% in the sludge. Nitrogen and phosphorus were released into the fermentation liquid. These released gases, however, showed no effect on PHA synthesis, making it possible for the fermentation liquid to be directly used for the production of PHA. 3-hydroxybutyrate (3HB) (73.5mmolC%), 3-hydroxyvalerate (3HV) (24.3mmolC%) and 3-hydroxy-2-methylvalerate (3H2MV) (2.2mmolC%) were the main constituents of the accumulated PHA. γ-*proteobacteria*, α-*proteobacteria*, and β-*proteobacteria* were shown to be the major microorganism in the PHA synthesis system (data analysed using 165 rRNA gene clone library).

The biosynthesis of PHA by *Alcaligenes* spp. and *Pseudomonas oleovorans* using different substrates has been studied (Santhanam and Sasidharan, 2010). Fructose, lactose, glucose, commercial sugar and n-octane were studied as different carbon sources. Medium with glucose carbon source gave rise to the maximum PHA content of 4.14g/L for *Alcaligenes eutrophus* while n-octane as carbon source produced 2.06g/L for *P. oleovorans*. Using FTIR spectroscopy analysis, C=O group was identified as the functional groups of the retrieved PHA granules.

An attempt was made to enhance the production of PHB by supplementing the carbon sources via a two-stage process and continuous feeding of ammonium chloride (Yeo et al., 2008). Gluconate and glucose were respectively used as carbon source in the first and second stages. The specific growth rate was maximized with the aid of gluconate while glucose was used to maximize PHB biosynthesis. In contrast to cultures in which glucose was used as the sole substrate, a 50% enhancement of PHB productivity was achieved when gluconate and glucose were consecutively fed. Also feeding the culture with a trace amount of NH_4Cl (0.03mmol/h) before the initial amount of NH_4Cl is used up enhanced PHB production compared to cultures containing glucose alone. A higher level of NADPH was observed in glucose-grown culture, during the NH_4Cl-exhausted PHB accumulation stage, compared to the gluconate-grown culture.

PHB has similarly been synthesized by a batch culture of *Alcaligenes latus*, American Type Culture Collection 29713, using sucrose as substrate (Grothe et al., 1999). Under optimum condition, the PHB yield was up to 63% of dry cell mass when cultured for 93 hours, with a mean biomass yield coefficient on sucrose of around 0.4kg/kg. Using ammonium sulphate or ammonium chloride as the source of nitrogen at pH 6.5 and temperature between 25^0C and 37^0C were found to give the optimum condition for PHB yield.

Lactose, a disaccharide of galactose and glucose, linked by a glycosidic bond has been useful as a carbon source for PHA synthesis. Whey is a cheap and sustainable source of lactose and it, as well as whey permeate, has been used as a carbon source by the following bacteria strains; *Hydrigenophaga pseudoflava* DSM 1034 and *Sinorhizobium melitoti* 41 (Povolo et al., 2003; Povolo et al., 2013), *Methylobacterium* sp. ZP24 (Yellore and Desai, 1998), *Bacillus megaterium* (Pandians et al., 2010), recombinant *Escherichia coli* (Ahn et al., 2000; Ahn et al., 2001), *Thermus thermophilus* HB8 (Pantazaki et al., 2009).

The major constituent of corn, sorghum, cassava, rice and potato is starch. It is made up of D-glucose units with α-1,4-glycosidic bonds. Glucose is the most extensively utilized carbon source for the industrial scale production of PHA. It is yielded upon enzymatic or acid hydrolysis of starch. Imperial Chemical Industries (ICI) produced commercial PHA using glucose derived from starch with *R. eutropha* (Bryom, 1992).

Fermentation of glucose was compared with xylose and arabinose fermentation using *Pseudomonas pseudoflava* (Bertrand et al., 1990). The conversion using glucose as a substitute is found higher (0.40gPHA/g) than using xylose and arabinose (0.17-0.19gPHA/g). The production of PHA when xylose and arabinose were utilized by the cells was also slower (four- to five-times) than when glucose was used. As a co-substrate with glucose, liquefied wood has also been used to incorporate 3-hydroxyvalerate units into the produced PHA (Koller et al., 2015).

In a recent study, PHA producing bacteria were isolated from diverse soil, dump sites and water samples. Media optimization was however required to improve the amount of PHA produced. Glucose and ammonium sulphate were found to be the best carbon and nitrogen source for PHA production. The

isolate exhibited innate capability to hydrolyze agricultural residue supplied as carbon source and used them for PHA production. Further, the optimum pH, temperature and time of recovery of PHA in *Bacillus thuringiensis* SBC4 was 7.0, 37°C and 48 h. The modified medium compounded from optimised parameters increased PHA yield, while FTIR analysis showed characteristic PHA peaks (Odeniyi and Adeola, 2017).

Commercial production of PHA

Only four PHA have been produced on a commercial scale and these are: poly[(*R*)-3-hydroxybutyrate] (PHB), poly[(*R*)-3-hydroxybutyrate-*co*-(R)-3-hydroxyvalerate] (PHBV), poly[(*R*)-3-hydroxybutyrate-*co*-4-hydroxybutyrate] (P3HB4HB), and poly[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyhexanoate] (PHBHHx). However, small-scale production of medium-chain-length (mcl) PHA has been done. The production of PHA generally involve several steps, these include: fermentation, separation of biomass from the broth, biomass drying, PHA extraction, drying, and packaging (Figure 2).

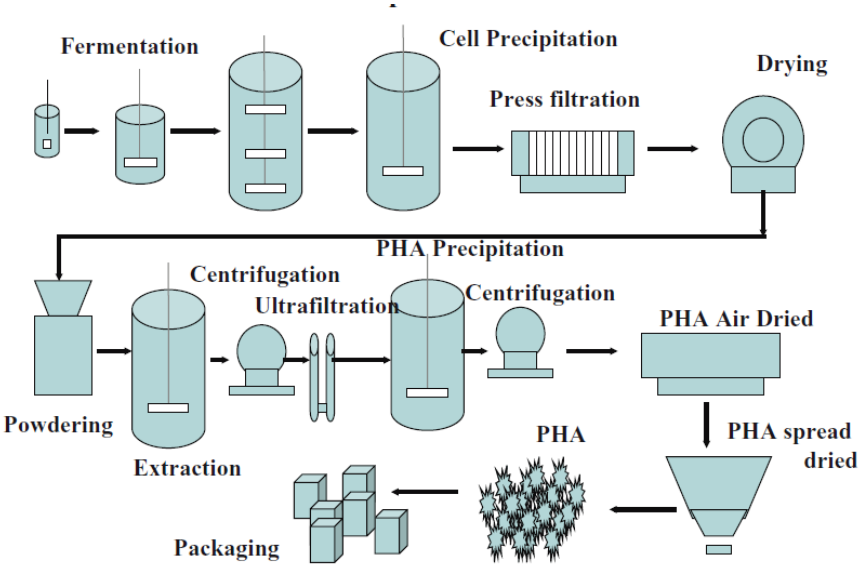

Figure 2. Polyhydroxyalkanoate production and extraction process

Nonato et al. (2001) suggested that PHB and related copolymers can be advantageously produced when the production is integrated into a sugarcane mill. In this set-up, the energy necessary for the production process is provided by biomass and the polymer produced at low cost in light of the available low-price carbon source and energy (Nonato et al. 2001).

The cost of production of PHA can also be ameliorated by blending with low cost materials such as starch and cellulose, while retaining the desirable characteristics of the polymer (Chen and Wu, 2005).

PHA as a drug delivery polymer

The primary objective of drug delivery systems is to increase the bioavailability and controlled release of drugs with respect to time and space. PHA materials have a huge potential in drug delivery applications (Francis et al., 2016). PHA microspheres or microcapsules have been used as drug carrier for the delivery of anaesthetics, hormones, steroids, antibiotics, anti-inflammatory agents, anticancer agents and vaccines (Nobes et al., 1998; Orts et al., 2008).

Shah et al. (2010) prepared an amphiphilic nanoparticle by an emulsification-solvent evaporation technique. The nanoparticles were prepared from diblock copolymer produced by chemically coupling poly(hydroxybutyrate-co-3-hydroxyvalerate) or poly(3-hydroxybutyrate-co-4-hydroxybutyrate) to monomethoxy poly(ethylene glycol) (mPEG), using transesterification reaction. The drug thymoquinone was incorporated into the nanoparticle and the drug release profile examined. Enzymatic degradation of the mPEG-coupled and non-coupled PHA nanoparticles was compared. The comparison showed that the nanoparticles aligned into an outer hydrophilic shell of mPEG attached to the interior hydrophobic core of PHA. It was, therefore, opined that, due to the fact that the amphiphilic nanoparticles contained the hydrophobic PHA portion embedded in the core, they could be safely used as carriers for the controlled re-

lease of different hydrophobic drugs.

Magnetic PHB nanoparticles to target cancer cells were prepared by Erdal et al. (2012). PHB polymer synthesized from *A. eutrophus* was used to coat iron oxide particles, using the multi-emulsion technique. Fourier Transform Infrared Spectrometry (FTIR), Electron Spin Resonance (ESR) and Vibrating Sample Magnetometer (VSM) and Scanning electron microscope (SEM) and Atomic force microscope (AFM) were respectively used to structurally, magnetically and morphologically characterize the nanoparticles. Etoposide (used as a drug) and Concanavalin-A (Con-A) (used to target cancer cells) were loaded onto the nanoparticles and the release kinetics of the drug evaluated.

Lee et al. (2011) demonstrated a method for the functionalization of hydrophobic nanocarrier surface via enzymatic polymerization. Coupling of hydrophobic surface of PHB nanoparticles with PHB chain grown from PHA synthase to which a specific ligand is attached was used to functionalize the nanoparticles. The ligand used, RDG4C, is specific to MDA-MB231 cancer cells as shown by the affinity of the functionalized PHA nanoparticles. This affinity indicates that targeting ability was conferred on the drug carrier due to the effective display of RDG4C on the PHB nanoparticles surface through enzymatic modification.

Poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) [P(HB-HO)] was used as a carrier in a system of targeted drug delivery (Zhang et al., 2010). Folic acid (FA) and doxorubicin (DOX) were respectively employed as targeting ligand and anticancer drug. The mean size, encapsulation efficiency and the drug loading capacity of the produced doxorubicin-loaded, folate-mediated P(HB-HO) nanoparticles (DOX/FA-PEG-P(HB-HO) nanoparticles) were respectively, 240nm, 83.5% and 29.6%. An *in vitro* intracellular uptake test showed the HeLa cells to be more efficient in taking up the DOX/FA-PEG-P(HB-HO) nanoparticles. Due to the uptake, the nanoparticles at IC_{50} of 0.87μm were more toxic to the HeLa cells compared to other treated cells. The nanoparticles also displayed a better efficacy against tumor growth in an in vivo observation. The final mean volume of the tumor (178.91 ± 17.43mm^3) was found to be of smaller size, compared to the normal saline control group (542 ± 45.19mm^3). The folate-mediated nanoparticles loaded with doxorubicin were shown to be effectively used in the targeted delivery of anticancer to folate receptor overexpressed tumor cells.

PHB and copolymer poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) nanoparticles were shown to be utilized for controlled intracellular drug release (Xiong et al., 2009). Rhodamine B isothiocyanate (RBITC) was used to examine the drug release properties of the prepared nanoparticles of PHB, PHBHHx and polylactic acid (PLA). The drug loading efficiency shown by the nanoparticles was more than 75%. Macrophage endocytosis gave rise to a controlled intracellular drug release that lasted up to 20 days for nanoparticles of PHB and PHBHHx. Nanoparticles of PLA and the free drug however released over 15 and 7 days, respectively.

Gangrade and Price (1991) examined the use of PHB and PHBV microspheres as carriers for the controlled delivery of progesterone. PHB-PHBV microspheres were well incorporated with more than 80% of the theoretical progesterone content. PHBV microspheres with 9% hydroxyvalerate had the slowest *in vitro* release due to their less porosity compared to microspheres of other polymers.

Rubomycin incorporated in absorbable polymeric PHB matrix in the microparticulate form has been studied for antitumor efficiency. Mice transplanted with Ehrlich ascitic carcinoma was used in the examination of the anti-tumor effect of the dosage form. The drug was shown to be effective in arresting the carcinoma proliferation and thus increased mice survival (Shishatskaya et al., 2008).

Lu et al. (2011) reported the use of PHA as an anticancer drug carrier. Phosphoinositide-3-kinase (P13K) inhibitor (TGX221) controlled release system was prepared and used to inhibit cancer cell lines growth. The controlled release system was PHA nanoparticle-based. The PHA-based nanoparticles gradually released TGX221 and significantly slowed growth of cancer cell lines compared to cancer cells treated with free drug or negative control.

Self-assembled amphiphilic copolymer nanoparticles loaded with cisplatin was examined for cisplatin efficacy and bioavailability (Shah et al., 2012). Coupling of poly (3-hydroxyvalerate-co-4hydroxybutyrate) with monomethoxypoly (ethylene glycol) through transesterification, using bis(2-ethylhexanoate) tin as catalyst, was used to synthesize an amphiphilic block polymer P(3HV-CO-4HB)-b-mPEG. The polymer was loaded with cisplatin and an *in vitro* drug release profile studied. The study showed a sustained drug release from the polymer hydrophobic core. Examination by transmission electron and confocal microscopy showed cisplatin-loaded nanoparticles to be well incorporated in the cancer cells. The

examination also showed that the growth of the cells was inhibited.

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(HB-co-HV)] rod impregnated with gentamycin or sulperazone was studied for in vitro antibiotic release. A sustained release of the antibiotics into aqueous solution for a period of two weeks was observed. Altering the hydroxyvalerate (HV) content of the copolymer changed the kinetics of drug release. A higher HV content (20%, compared with 7% or 14%) prolonged the controlled release of sulperazone to 60 days (Gursel et al., 2002).

The release of gentamycin incorporated into P(HB-co-HV) discs was measured over time. Copolymers containing 12% HV and 8% HV at 2:1 or 5:1 (w/w) ratio were compared and the copolymer with higher HV content found to release more gentamycin into solution (Rossi et al., 2004).

Different PHA polymers and copolymer possess different drug release potentials. An example of this was demonstrated by Wang et al. (2003). It was shown that tamsulosin incorporated with poly(hydroxyhexanoate-co-hydroxyoctanoate) (P(HHx-co-HO)) copolymer facilitated the permeation of drug into the skin. PHB has higher crystallinity and poor skin adherence in the studied system and was, therefore, less suitable for the task.

Tetracycline was incorporated into P(3HB-co-3HV), the 3HV content varied and the copolymer investigated for tetracycline controlled release (Sendil et al., 1999). Tetracycline was loaded in both the neutral and acidic forms into the PHBV microspheres and microcapsules. In vitro release profile of the systems was then carried out. The release was found to be total before degradation set in.

Tablet preparations intended for controlled release of 7-hydroxyethyltheophylline were embedded within the P(3HB) matrix (William and Martin, 2005). The tablets were prepared by mixing and compressing the polymer and the drug. However, the number of investigations reported in literature on PHA tablet formulations is significantly lower than that of biodegradable implants and microencapsulation. This low interest in the usage of PHAs in tablet formulation might be due to relative high cost of PHAs which dwarfs the possible benefits.

The release of fluorescein, and dextrans labelled with fluorescein, from tablets prepared with poly (3HB-co-3HV) prepared by direct compression method was investigated (Gould et al., 1987). The rate of drug release was found to significantly increase with increase in drug loading and with increase in valerate in the copolymer (Korsatko et al., 1983). The addition of microcrystalline cellulose or lactose also caused an increase in release rate (Gould et al., 1987).

Further, blends of rigid P(3HB) and flexible P(3HO) were investigated as a matrix for drug-eluting coronary stents. The local delivery of antithrombotic agents can significantly reduce the risk of reoccurrence of the arterial blockage after stent implantation (Basnett et al., 2013). Hence, aspirin was loaded into the matrix of the PHA blend via solvent casting, and the drug was uniformly distributed throughout the matrix. Only a weak burst release of the drug (less than 10% of total load) was observed in the first 24 hours of *in vitro* release studies. Following the initial burst release, aspirin was released at a relatively constant rate within 20 days and practically full release was achieved after 30 days. It is of interest to note that embedding the aspirin in the hydrophobic PHA matrix, thereby increasing its stability, significantly increased delivery of approximately 60% of aspirin in its therapeutically active form.

In an *in vitro* study of antibiotic release in an aqueous solution, P(HB-*co*-HV) rods impregnated with either gentamicin or Sulperazone sustained drug release for 2 weeks. Also, using a higher HV content copolymer (20% HV, compared to 7% or 14% HV), a sustained release of Sulperazone for over 60 days was achieved. Kinetics of release was altered by changing the amount of drug loading. Higher levels of cumulative release were obtained using a copolymer with higher HV content (Gursel et al., 2002). Table 1 presents a survey of drug release from some PHA matrices (Bringham and Sinskey, 2012).

Table 1. Survey of Drug Release Studies from PHA Matrices

Polymer/composite	Drug	References
P(HB-*co*-HV)	Tetracycline	Sendil et al., 1999
PHB	Rifampicin	Kassab et al., 1997
P(HB-*co*-HV)	Sulbactam-cefoperazone	Yagmurlu et al., 1999
P(HB-*co*-HV)	Gentamicin or Sulperazone	Gursel et al., 2002
P(HB-*co*-HV)/wollastonite composite	Gentamicin	Li et al., 2005
P(HB-*co*-HV)	Gentamicin	Rossi et al., 2004
P(HB-*co*-HHx)	Rhodamine B isothiocyanate	Yao et al., 2008
PHB	Rubomycin	Shishatskaya et al., 2008

Gentamicin was incorporated into P(HB-*co*-HV) discs, and drug release was measured over time. Polymer containing higher HV content displayed a higher degree of drug release into solution (12% 3HV v. 8% HV). These P(HB-*co*-HV) discs containing gentamicin incubated in normal human blood samples displayed no toxicity as there were no proliferation of white blood cells, red blood cells or platelets (Rossi et al., 2004)

Lower crystallinity PHA has been suggested for better timed release of drug into the surrounding tissue as studies have shown that the release of antibiotics, such as rifampicin and tetracycline, from PHB microspheres results in a too rapid release (Zinn et al., 2001). For example, on mixing tamulosin with poly(hydroxyhexanoate-*co*-hydroxyoctanoate (P(HHx*co*-HO)) polymer, permeation of the drug through the polymer into skin was facilitated. However, PHB was less suited for this due to both its higher crystallinity and its inability to adhere to skin in the system tested (Wang et al., 2003). P(3HB-*co*-4HB) polymer has also been shown to be effective at releasing drug in solution (Tuercin et al., 2001).

Microspheres of P(3HB) have been prepared using the spray drying technique, a technique rarely used for the production of PHA particles (Kim et al., 2000). The method resulted in the formation of microspheres of smaller size range (0.6-1.1μm as opposed to 5-100 μm prepared by the solvent evaporation method), uniform size distribution and high porosity, which results in higher drug loading. Thus *in vitro* studies with P(3HB) and P(3HB-3HHx) nanoparticles (150-250 nm) showed an efficient internalization into macrophage without affecting cell viability. Sustained release of the drug was achieved over a period of more than 20 days (Xiong et al., 2010). PHAs nanospheres were used for encapsulating TGX221, a PI3K inhibitor, blocking proliferation of cancer cells by the same research group. Drug-loaded nanoparticles directly delivered into the cancerous cells improved drug delivery and led to inhibition of cancer cells (Lu et al., 2011).

PHA nanoparticles have been loaded with a broad range of drugs to evaluate them for drug administration in various illnesses. Also, by attaching targeting moieties the particles have been modified to improve drug delivery. As mentioned earlier, one of the first studies involved the use of PHB and PHBV (9 and 24% of HV units) for the preparation of microspheres loaded with progesterone (Gangade and Price, 1991). The encapsulation efficiency for these systems was relatively high with more than 80% of the theoretical amount of drug being successfully loaded in all cases.

Another study involved the preparation of nanoparticles from PHB and PHBHx (containing 12% of HHx units) and a comparison against poly(-lactic acid) nanoparticles. No toxicity was observed for the PHA nanoparticles in the concentration range from 1 to 1000 μgmL^{-1}. A comparison of drug release profiles of PHA nanoparticles and poly(lactic acid) nanoparticles shows that slower release was obtained in the case of former systems.

Encapsulation of a drug in nanoparticles seek to extend the drug half-life and thereby improve dosing regimen, improve bioavailability or to enhance the transport properties of the drug. These goals are achieved using PHA-based nanoparticles in anticancer therapy concerning TGX221, an inhibitor of phosphoinositide-3-kinases (Lu et al., 2011). Here, nanoparticles with average size of around 200nm were prepared from PHB, PHBV (5% HV) and PHBHx (12% HHx). These systems demonstrated that entrapment of TGX221, a PI3K inhibitor, extended its bioavailability and *in vivo* half-life. Also, Ellipticine was encapsulated in nanoparticles formed from PHBV containing 5, 11 and 15% of HV units

(Masood et al., 2013). The administration of this drug to the A549 cancer cell line doubled the effectiveness of the inhibition of cell growth compared to free ellipticine by enhancing the bioavailability. Rapamicyn demonstrated better anti-proliferative effect after loading into PHBHx nanoparticles (Lu et al., 2014). Furthermore, PHA nanoparticles (PHBV containing 12 and 50% of HV units) were successfully used in administering porphyrin 5,10,15,20-tetrakis(4-hydroxyphenyl)-21*H*,23*H*-porphine for anticancer photodynamic therapy (Pramual et al., 2016).

CONCLUSION

There has been an increased interest in PHA use in the delivery of drugs and other medical materials due to their biodegradability, biocompatibility and non-toxicity. Industrial scale production cost of PHA has always been the impediment to the use of the polymer. Extensive efforts have been employed by researchers to drive down the cost by utilizing cheap and renewable carbon sources, which account for the bulk of the cost. This has made the replacement of synthetic plastics with these biodegradable ones promising. The appealing properties of the PHA have also attracted their use in cancer therapy and controlled release formulations. Researches have been carried out, with success, on their use in the delivery of antitumor drugs, steroids and other biologically active substances. The results of these works are a pointer to a potential revolution in the use of PHA for drug delivery.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

Ahn, W. S., Park, S. J., & Lee, S. Y. (2000). Production of poly(3-hydroxybutyrate) by fed-batch culture of recombinant *Escherichia coli* with a highly concentrated whey solution. *Applied and Environmental Microbiology, 66*(8), 3624-3627.

Ahn, W. S., Park, S. J., & Lee, S. Y. (2001). Production of poly(3-hydroxybutyrate) from whey by cell recycle fed-batch culture of recombinant *Escherichia coli. Biotechnology Letters, 23*, 235-240.

Alsafadi, D. & Al-Mashaqbeh, O. (2017). A one-stage cultivation process for the production of poly-3-(hydroxybutyrate-*co*-hydroxyvalerate) from olive mill wastewater by *Haloferax mediterranei. New Biotechnology, 34*, 47-53.

Aremu, M. O., Layokun, S. K., & Solomon, B. O. (2010). Production of poly (3-hydroxybutyrate) from cassava starch hydrolysate by *Pseudomonas aeruginosa* NCIB 950. *American Journal of Scientific and Industrial Research, 1*(3), 421–426.

Ataei, S. A., Vasheghani-Farahani, E., Shojaosadati, S. A., & Tehrani, H. A. (2008). Isolation of PHA-producing bacteria from date syrup waste. *Macromolecular Symposia, 269*, 11-16.

Basnett. P., Ching, K. Y., Stolz, M., Knowles, J. C., Boccaccini, A. R., Smith, C., Locke, I. C., & Roy, I. (2013). Aspirin-loaded P(3HO)/P(3HB) blend films: Potential materials for biodegradable drug-eluting stents. *Bioinspired, Biomimetic and Nanobiomaterials, 2*,141-153.

Beneke, C. E., Viljoen, A. M., & Hamman, J. H. (2009). Polymeric plant-derived excipients in drug delivery. *Molecules,* 14, 2602-2620.

Bertrand J. L., Ramsay B. A., Ramsay J. A., & Chavarie C. (1990). Biosynthesis of poly-β-hydroxyalkanoate from pentoses by *Pseudomonas pseudoflava*. *Applied and Environmental Microbiology*, *56*(10), 3133-38.

Bryom, D. (1992). Production of poly-β-hydroxybutyrate:poly-β-hydroxyvalerate copolymers. *FEMS Microbiol Rev, 103*, 247-250.

Cavalheiro, J. M. B. T., Almeida, M. C. M. D., Grandfils, C., & Fonseca, M. M. R. (2009). Poly(3-hydroxybutyrate) production by *Cupriavidus necator* using waste glycerol. *Process Biochemistry, 44*, 509–515.

Chen, C. W., Don, T. M., & Yen, H. F. (2006). Enzymatic extruded starch as a carbon source for the production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by *Haloferax mediterranei*. *Process Biochemistry, 41*, 2289–2296.

Chen, G. Q., & Wu, Q. (2005). Polyhydroxyalkanoates as tissue engineering materials. *Biomaterials, 26*, 6565–6578.

Erdal, E., Kavaz, D., Sam, M., Demirbilek, M., Demirbilek, M. E., Saglam, N., & Denkbas, E. B. (2012). Preparation and characterization of magnetically responsive bacterial polyester based nanospheres for cancer therapy. *Journal of Biomedicine and Nanotechnology,* 8(5), 800-808.

Errico, C., Bartoli, C., Chiellini, F., & Chiellini, E. (2009). Poly(hydroxyalkanoates)-based polymeric nanoparticles for drug delivery. *Journal of Biomedicine and Biotechnology,* 1-10.

Flechter, A. (1993). In: Plastics from Bacteria and for Bacteria: PHA as natural, biodegradable polyesters. *Springer Verlag, New York,* 77–93.

Francis L., Meng D., Locke I.C., Knowles J.C., Mordan N., Salih V. & Roy I. (2016). Novel poly (3-hydroxybutyrate) composite films containing bioactive glass nanoparticles for wound healing applications. *Polymer International,* 65: 661–674.

Gangrade, N., & Price, J. C. (1991). Poly(hydroxybutyrate-hydroxyvalerate) microspheres containing progesterone: preparation, morphology and release properties. *Journal of Microencapsulation*, *8*(2), 185–202.

Ganzeveld, K. J., Hagen, A. V., Agteren, M. H., Koning, W., & Uiterkamp, A. J. M. S. (1999). Upgrading of organic waste: production of the copolymer poly-3-hydroxybutyrate-co-valerate by *Ralstonia eutrophus* with organic waste as sole carbon source. *Journal of Cleaner Prod*, *7*, 413–419.

Grothe, E., Moo-Young, M., & Christi, Y. (1999). Fermentationn optimization for the production of poly(β-hydroxybutyric acid) microbial thermoplastic. *Enzyme Microbiology and Technology, 25*, 132-141.

Gursel, I., Yagmurlu, F., Korkusuz, F., & Hasirci, V. (2002). In vitro antibiotics release from poly(3-hydroxybutyrate-co-3-hydroxyvalerate) rods. *Journal of Microencapsulation, 19*, 153-64.

Haas, R., Jin, B., & Zepf, F. T. (2008). Production of poly(3-hydroxybutyrate) from waste potato starch. *Biosciences Biotechnology and Biochemistry, 72*(1), 253-256.

Jiang, Y., Songa, X., Gonga, L., Li, P., Dai, C., & Shao, W. (2008). High pol3hydroxybutyrate production by *Pseudomonas fluorescens* A2a5 from inexpensive substrates. *Enzyme Microbiology Technology, 42*, 167–172.

Jiang, Y., Chen, Y., & Zheng, X. (2009). Efficient polyhydroxyalkanoates production from a waste-activated sludge alkaline fermentation liquid by activated sludge submitted to the aerobic feeding and discharge process. *Environmental Science and Technology, 43*, 7734–7741.

Kassab, A. C., Xu, K., Denkbas, E. B., Dou, Y., Zhao, S., & Piskin, E. (1997). Rifampicin carrying polyhydroxybutyrate microspheres as a potential chemoembolization agent. *Journal of Biomaterial Science Polymer Ed, 8*(12), 947-61.

Kim, G. J., Bang, K. H., Kim, Y. B., & Rhee, Y. H. (2000). Preparation and characterization of native poly(3-hydroxybutyrate) microspheres from *Ralstonia eutropha*. *Biotechnology Letters, 22*, 1487-1492.

Koller, M., de Sousa Dias, M. M., Rodriguez-Contreras, A., Kunaver, M., Zagar, E., & Braunegg, G. (2015). Liquefied wood as an inexpensive precursor-feedstock for bio-mediated incorporation of (R)-3-hydroxyvalerate into polyhydroxyalkanoates. *Materials, 8*, 6543-6557.

Korsatko, W., Wabnegg, B., & Tillian, H. M. (1983). Poly-D-(-)-3-hydroxybutyric acid: a biodegradable carrier for long term medication dosage. II. Comm.: the biodegradation in animal organism and *in-vitro - in-vivo* correlation of the liberation of pharmaceuticals from parenteral matrix retard tablets. *Pharmacy Industry*, *45*(10), 1004–1007.

Lee, J., Jung, S., Park, C., Kim, H., Batt, C. A., & Kim, Y. (2011). Tumor-specific hybrid polyhydroxybutyrate nanoparticle: surface modification of nanoparticle by enzymatically synthesized functional block copolymer. *Bioorganic and Medicinal Chemistry Letters*, *21*(10), 2941–2944.

Li, H., & Chang, J. (2005). Preparation, characterization and in vitro release of gentamicin from PHBV/wollastonite composite microspheres. *Journal of Control Release, 107*(3),463-73.

Lu, X.-Y., Ciraolo, E., Stefenia, R., Chen, G.-Q., Zhang, Y., & Hirsch, E. (2011). Sustained release of PI3K inhibitor from PHA nanoparticles and *in vitro* growth inhibition of cancer cell lines. *Applied Microbiology Biotechnology*, *89*(5), 1423–1433.

Lu, X. Y., Li, M. C., Zhu, X. L., Fan, F., Wang, L. L., & Ma, J. G. (2014). Microbial synthesized biodegradable PHBHHxPEG hybrid copolymer as an efficient intracellular delivery nanocarrier for kinase inhibitor. *BMC Biotechnology, 14*(4), 1-10.

Martinez, G. A., Bertin, L., Scoma, A., Rebecchi, S., Braunegg, G., & Fava, F. (2015). Production of polyhydroxyalkanoates from dephenolised and fermented olive mill wastewaters by employing a pure culture of *Cupriavidus necator. Biochemical Engineering Journal. 97*, 92-100.

Masood, F., Chen, P., Yasin, T., Fatima, N., Hasan, F., & Hameed, A. (2013). Encapsulation of Ellipticine in poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) based nanoparticles and its *in vitro* application. *Material Science Engineering C,* 33,1054–1060.

Moita, R., Freches, A., Lemos, & P. C. (2014). Crude glycerol as feedstock for polyhydroxyalkanoates production by mixed microbial cultures. *Water Resources, 58*, 9-20.

Moore, G. F. & Saunders, S. M. (1998). Advances in Biodegradable Polymers, iSmithers Rapra Publishing, Akron, Ohio.

Nobes, G. A. R., Marchessault, R. H., & Maysinger, D. (1998). Polyhydroxyalkanoates: materials for delivery systems. *Drug Delivery, 5*(3), 167-177.

Nonato, R. V., Mantelatto, P. E., & Rossell, C. E. V. (2001). Integrated production of biodegradable plastic, sugar and ethanol. *Applied Microbiology and Biotechnology, 57*, 1–5.

Odeniyi, O. A., & Adeola O. J. (2017). Production and characterization of polyhydroxyalkanoic acid from *Bacillus thuringiensis* using different carbon substrates. *International Journal of Biological Macromolecules, 104*, 407–413.

Orts, W. J., Nobes, G. A. R., Kawada, J., Nguyen, S., Yu, G. E. & Ravenelle, F. (2008). Poly(hydroxyalkanoates): biorefinery polymers with a whole range of applications. The work of Robert H. Marchessault. *Canadian Journal of Chemistry, 86*(6), 628-640.

Pandians, R., Deepak, V., Kalishwaralal, K., Rameshkumar, N., Jeyaraj, M., & Gurunathan, S. (2010). Optimization and fed-batch production of PHB utilizing dairy waste and sea water as nutrient sources by *Bacillus megaterium* SRKP-3. *Bioresources Technology, 101*(2), 705-711.

Pantazaki, A. A., Papaneophytou, C. P., Pritsa, A. G., Liakopoulus-Kyriakides, M. & Kyriakidis, D. A. (2009). Production of polyhydroxyalkanoates from whey by *Thermus thermophiles* HB8. *Process Biochemistry, 44*, 847-853.

Povolo, S., & Casella, B. (2003). Bacterial production of PHA from lactose and cheese whey permeate. *Macromolecules Symposium, 197*, 1-10.

Povolo, S., Romanelli, M. G., Basaglia, M., Llieva, V. I., Corti, A., Morelli, A., Chiellini, E., & Casella, S. (2013). Polyhydroxyalkanoate biosynthesis by *Hydrogenophaga pseudoflava* DSM1034 from structurally unrelated carbon sources. *N Biotechnology, 30*(6), 629-634.

Pramual, S., Assavanig, A., Bergkvist, M., Batt, C. A., Sunintaboon, P., Lirdprapamongkol, K.,….Niamsiri, N. (2016). Development and characterization of bioderived polyhydroxyalkanoate nanoparticles as a delivery system for hydrophobic photodynamic therapy agents. *J Mater SciMater Med* 27:40.

Rossi, S., Azghani, A. O., & Omri, A. (2004). Antimicrobial efficacy of a new antibiotic-loaded poly(hydroxybutyric-co-hydroxyvaleric acid) controlled release system. *Journal of Antimicrobial Chemotherapy, 54*(6), 1013-1018.

Sandhya, M., Aravind, J., & Kanmani, P. (2013). Production of polyhydroxyalkanoates from *Ralstonia eutropha* using paddy straw as cheap substrate. *International Journal of Environmental Science Technology, 10*, 47-54.

Santhanam, A., & Sasidharan, S. (2010). Microbial production of polyhydroxyalkanotes (PHA) from *Alcaligens spp*. and *Pseudomonas oleovorans* using different carbon source. *African Journal of Biotechnology, 9*, 3144–3150.

Sendil, D., Gursel, I., Wise, D. L., & Hasirci, V. (1999). Antibiotics release from biodegradable PHBV microparticles. *Journal of Controlled Release, 59*(2), 207-217.

Shah, M., Naseer, M. I., Choi, M. H., Kim, M. O., & Yoon, S. C. (2010). Ampiphilic PHA-mPEG copolymeric nanocontainers for drug delivery: preparation, characterization and in vitro evaluation. *International Journal of Pharmaceutics, 400*(1-2), 165-175.

Shah, M., Ullah, N., Choi, M. H., Kim, M. O., & Yoon, S.C. (2012). Amorphous amphiphilic P(3HV-co-4HB)-b-mPEG block copolymer synthesized from bacterial copolyester via melt transesterification: nanoparticle preparation, cisplatin loading for cancer therapy and *in vitro* evaluation. *European Journal of Pharmaceutics and Biopharmaceutics*, *80*(3), 518–527.

Shishatskaya, E. I., Goreva, A. V., Voinova, O. N., Inzhevatkin, E. V., Khlebopros, R. G. & Volova, T. G. (2008). Evaluation of antitumor activity of rubomycin deposited in absorbable polymeric microparticles. *Bulletin of Experimental Biology and Medicine*, *145*(3), 358–361.

Tuercin, F., Gursel, I., & Hasirci V. (2001). Biodegradable polyhydroxyalkanoate implants for osteomyelitis therapy: *in vitro* antibiotic release. *Journal of Biomaterial Science Polymer Edition*, *12*(2): 195-207.

Urtuvia, V., Villegas, P. Gonzalez, M., & Seeger, M. (2014). Bacteria production of biodegradable plastics polyhydroxyalkanoates. *International Journal of Biological Macromolecules, 70*, 208–213.

Verlinden, R. A. J., Hill, D. J., Kenward, M. A., Williams, C. D., & Radecka, I. (2007). Bacterial synthesis of biodegradable polyhydroxyalkanoates. *Journal of Applied Microbiology, 102*, 1437-1449.

Wang, Z., Itoh, Y., Hosaka, Y., Kobayashi, I., Nakano, Y., Maeda, I.,….Yagi, K. (2003). Mechanism of enhancement effect of dendrimer on transdermal drug permeation through polyhydroxyalkanoate matrix. *Journal of Biosciences and Bioengineering, 96*(6), 537-540.

Williams, S. F., & Martin, D. P. (2005). Applications of polyhydroxyalkanoates (PHA) in medicine and pharmacy. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

Xiong, Y., Yao, Y., Zhan, X., & Chen, G. (2009). Application of polyhydroxyalkanoate nanoparticles as intracellular sustained drug release vectors. *Journal of Biomedical Sciences, 21*, 127-140.

Yagmurlu, M. F., Korkusuz, F., Gursel, I., Korkusuz, P., Ors, U., & Hasirci, V. (1999). Sulbactam-cefoperazone polyhydroxybutyrate-cohydroxyvalerate (PHBV) local antibiotic delivery system: in vivo effectiveness and biocompatibility in the treatment of implant-related experimental osteomyelitis. *Journal of Biomedicine and Material Research, 46*(4), 494-503.

Yao, Y. C., Zhan, X. Y., Zhang, J., Zhou, C. H., Wang, Z. H., Xiong, Y. C…..Chen, G. Q. (2008). A specific drug targeting system based on polyhydroxyalkanoate granule binding protein PhaP fused with targeted cell ligands. *Biomaterials 29*(36): 4823-30.

Yellore, V., & Desai, A. (1998). Production of poly-3-hydroxybutyrate from lactose and whey by *Methylobacterium* sp. ZP24. *Letters Applied Microbiology,* 26(6), 391-394.

Yeo, J. S., Park, J. Y., Yeom, S. H., & Yo, Y. J. (2008). Enhancement of poly-3-hydroxybutyrate productivity by the two stage supplementation of carbon sources and continuous feeding of NH4Cl. *Biotechnology and Bioprocess Engineering*, *13*, 14–24.

Yilmaz, M., & Beyatli, Y. (2005). Poly-β-hydroxybutyrate (PHB) production by a *Bacillus cereus* M5 strain in sugarbeet molasses. *Zuckerindustrie, 130*, 109–112.

Zhang, C., Zhao, L. Q., Dong, Y. F., Zhang, X. Y., Lin, J., & Chen, Z. (2010). Folate-mediated poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) nanoparticles for targeting drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *76*(1), 10–16.

Zinn, M., Witholt, B., & Egli, T. (2001). Occurrence, synthesis and medical application of bacterial polyhydroxyalkanoate. *Advanced Drug Delivery Reviews, 53*(1), 5–21.