Co-utilization of Crude Glycerol and Biowastes for Producing Polyhydroxyalkanoates

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Abstract Polyhydroxyalkanoate (PHA) production by *Bacillus thuringiensis* EGU45 and defined mixed culture of *Bacillus* spp. were studied by using crude glycerol (CG) and hydrolyzed biowastes as feed material. Hydrolysates from onion peels (OP), potato peels, pea-shells (PS), apple pomace 2% total solids obtained with defined mixed hydrolytic cultures (MHC2) were inoculated with *B. thuringiensis* EGU45 and defined mixed bacterial cultures (5MC1), which produced PHA at the rate of 40–350 and 65–450 mg/L, respectively. Addition of CG (1%, v/v) to these hydrolysates resulted in 1.8-fold and 4.5-fold enhancement in PHA production from OP by *B. thuringiensis* EGU45 and 5MC1, respectively. Co-utilization of OP and PS (in 2:1 ratio) supplemented with CG (1%, v/v) by *B. thuringiensis* EGU45 resulted in 2-fold increase in PHA production in comparison to OP + CG. This co-metabolism of OP and PS also enabled PHA co-polymer production (1300 mg/L), having an enhanced HV content of 21.2% (w/w).

Keywords *Bacillus* · Crude glycerol · Biowastes · Vegetable wastes · Mixed hydrolytic culture

Introduction

Petroleum based synthetic plastics are destroying fragile ecosystems. These plastics cause serious threat to human health and the environment as they are non-biodegradable in nature. Replacement of these polymers with biodegradable polymers has gained major attention in recent years [1, 2]. Among the biopolymers, polyhydroxyalkanoates (PHAs) have gained importance due to their biodegradable nature and the fact that these can be produced from natural and renewable resources. They have physical and chemical properties similar to petroleum based synthetic plastics [3]. PHAs have attracted the attention of many researchers due to their non-polluting properties. PHAs are produced by bacteria under stress conditions. Depending upon the type of substrates most bacteria produce homo- and co-polymers of PHAs [3]. Homopolymers (PHB) being brittle are restricted in their applications. Thus, there is the need for producing ductile co-polymers, which have the potential for medical applications and also have high commercial value [1, 2]. However, there are many challenges in customizing PHA compositions such as varying in monomeric compositions, molecular weight, tensile strength and elongation [4]. These modifications for improving PHA strength can be achieved through synthetic biochemical approaches [5].

PHA co-polymers production can be achieved by using biowastes as feed. As bacteria have an ability to exploit a variety of complex wastes, these turn out to be an obvious choice as feed material. Hydrolysis of these complex biowastes is essential for the release of intermediates, which will help in PHA co-polymer production [3, 6–9]. Although many biowastes have been used as feed for PHA production, however, in recent years effluent from biodiesel industry has gained attention as it

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is produced in large quantities. The effluent released from biodiesel industry is composed of 70% as crude glycerol (CG) among other impurities [10, 11]. CG has been shown as feed for PHA production by diverse bacterial species [1, 10]. On the other hand, biowastes originating from municipal markets such as apple pomace (AP), potato peels (PP), pea-shells (PS) and onion peels (OP) have been also shown to act as feed material for PHA producing bacteria [3, 8, 9]. Although co-metabolism of: (1) market wastes and glucose, and (2) CG, glucose and propionic acid combinations have been helpful in producing PHA co-polymers [1–3, 10]. However, PHA copolymer production by co-utilization of municipal market wastes and biodiesel industry wastes have not been demonstrated so far. In this study, we have shown that PHA co-polymers can be produced from municipal market wastes and CG in different combinations by *Bacillus thuringiensis* and defined mixed microbial cultures. It also resulted in enhanced 3-hydroxyvalerate (3HV) content in the co-polymer of PHA.

Materials and Methods

Organism and Its Growth Parameters

Bacterial strains used in this study were obtained from our laboratory stock. Different bacteria were used for preparing: (a) mixed hydrolytic bacterial culture (designated as MHC-2) constituted of *Bacillus sphaericus* strain EGU542; *B. thuringiensis* strain EGU378; *Bacillus* sp. strains EGU85, EGU367 and EGU447; and *Proteus mirabilis* strain EGU30, and (b) defined mixed microbial culture (designated as 5MC1) for producing PHA was constituted of *Bacillus cereus* strains EGU3, EGU43, EGU44, and EGU520, and *B. thuringiensis* strain EGU45 [9]. These bacterial strains were grown on nutrient broth (NB) (13 g/L) and incubated at 37 °C at 200 rev/min for a period of 16–20 h. The cultures of bacterial strains so prepared were used to inoculate media at the rate of 10 µg cellular protein/mL [3].

Preparation of Biowaste Hydrolysate

Biowastes—PS, OP, AP and PP were collected from municipal market of Delhi. These wastes were cut into 1–3 mm pieces, and mixed with 250 mL of distilled water in 300 mL BOD bottles to make a slurry (total solid, TS—2%) as described earlier [3]. Each slurry was inoculated individually with MHC2, to a concentration of 100 µg cell protein/mL. Digestion of biowaste slurries was performed at 37 °C for 48 h [3].

PHA Production on Waste Hydrolysate

200 mL of PS, PP, AP or OP slurry (2%, TS) hydrolyzed with MHC2 was filtered through 0.45 µm Whatman filter and used for PHA production. The pH of the hydrolysates was set at 7.2 and inoculated with *B. thuringiensis* EGU45 and 5MC1 at the concentration of 10 µg cell protein/mL of feed [3]. These hydrolysates were supplemented with crude glycerol (CG), (1%, v/v). The hydrolysates of PS, PP, AP, OP not supplemented with CG were used as controls. Subsequently, MHC2 hydrolysates of OP were mixed with AP, PP or PS in three different ratios of 1:2, 1:1 and 2:1. 100 mL of each of these mixed hydrolysates supplemented with or without CG (1%, v/v) was used as feed for *B. thuringiensis* EGU45 and 5MC1. The PHA production was monitored for 48 h of incubation at 37 °C and 200 rev/min.

Analytical Methods

PHA Analysis

100 mL aliquots of bacterial cultures were used to estimate the dry cell mass (DCM) and PHA production as described previously [1, 3]. The polymers were also analyzed for their monomeric composition using GC fitted with DB-5 (fused silica with 5% phenylpolydimethylsiloxane) column (30 m × 0.32 mm × 0.25 µm) [1].

Results

Utilization of biowastes such as AP, PP, PS and OP has been shown for producing PHA and its co-polymers. Supplementation of the biowaste with glucose proved helpful in further improving PHA production and its composition [3]. As CG is now available as waste from biodiesel industry hence, we explored the possibilities of its co-metabolism with these biowastes.

Influence of CG on PHA Production from Biowastes

B. thuringiensis EGU45 grows well on slurries of different biowastes such that the DCM of 720 mg/L on AP to 3590 mg/L on OP was recorded (Table 1). *B. thuringiensis* EGU45 could produce 40–350 mg PHA/L on slurries containing 2%, TS of 4 different biowastes: AP, PS, PP, OP. PHA constituted 2–17% of the total DCM. OP as feed proved to be the best in terms of DCM (3590 mg/L), and PHA (350 mg/L) production by *B. thuringiensis* EGU45. Addition of CG at the rate of 1%, v/v to these biowastes proved effective in enhancing DCM and PHA production. The influence of CG on PHA production by *B.*

Table 1 Influence of crude glycerol on bacterial polyhydroxyalkanoate production from biowastes

Feed	Bacterial culture									
	<i>Bacillus thuringiensis</i> EGU45					Mixed culture				
	DCM (mg/L)	PHA (mg/L)	%	Mol%		DCM (mg/L)	PHA (mg/L)	%	Mol%	
				HB	HV				HB	HV
Apple pomace (AP)	720	120	17	100	0	600	120	18	93	7
AP + 1% CG	820	130	16	55	45	1850	200	10	65	35
Potato peels (PP)	1040	40	2	92	8	1500	120	11	97	3
PP + 1%CG	2340	150	6	85	15	1700	105	5	75	25
Pea-shells (PS)	1200	70	6	98	2	850	65	8	95	5
PS + 1% crude glycerol (CG) (v/v)	1230	90	6	81	19	1700	455	26	72	28
Onion peels (OP)	3590	350	8	83	17	1900	450	23	91	9
OP + 1% CG	5400	630	11	28	72	3400	2040	60	70	30

PS pea-shells, PP potato peels, AP apple pomace, OP onion peels, CG crude glycerol, DCM dry cell mass, PHA polyhydroxyalkanoate, HB hydroxybutyrate, HV hydroxyvalerate

thuringiensis EGU45 was: 1.08-fold on AP, 1.28-fold on PS, 1.8-fold on OP and 3.75-fold on PP. *B. thuringiensis* EGU45 didn't produce HV on AP as feed, whereas it produced, co-polymers of PHA with the rest of the feeds. The addition of CG greatly influenced PHA co-polymers composition, where HV content varied from 15 to 72 mol%. With AP as feed HV content improved from nil in AP alone to 45 mol% in AP + CG as feed. Here, OP turned out to be the best feed either alone or along with CG, producing 5400 mg/L of DCM and 630 mg/L of PHA having 72 mol% HV (Table 1).

On the other hand, defined mixed bacterial culture (5MC1) was found to produce PHA in a significant quantity. Hydrolysates of AP, PP, PS and OP with MHC2 resulted in a PHA content of 65–450 mg/L of the total DCM recorded. PHA constituted 8–23% of the total yield (Table 1). Addition of CG to the biowastes slurries resulted in higher DCM (1700–3400 mg/L) and PHA (200–2040 mg/L). On individual basis, PHA yield improved 1.67-fold on AP, 7-fold on PS, 4.53-fold on OP. There was a decline in PHA yield on addition of CG to PP. In comparison to *B. thuringiensis* EGU45, PHA co-polymer production was observed with all the biowastes treated with mixed bacterial culture: 5-fold on AP + CG, 8.3-fold on PP + CG, 5.6-fold on PS and 3.3-fold on OP + CG compared to their respective individual feeds (Table 1). In this case also OP was found to be the suitable feed material for PHA and its co-polymer production, which was observed to vary up to 450 mg/L on OP with a DCM of 1900 mg/L. Supplementation of CG (1%, v/v) to the biowastes (OP) feed resulted in an enhanced DCM of 3400 mg/L. PHA production also increased up to 2040 mg/L, which is equivalent to a yield of 60%. The

supplementation of CG to other biowastes also resulted in enhanced bacterial growth in the range of 1700–1850 mg/L. PHA content varied from 105 to 455 mg/L. Although addition of CG (1%, v/v) proved helpful in improving DCM and PHA production, however, it negatively influenced the PHA co-polymer composition. The HV mol% was found to decrease from 72 mol% with *B. thuringiensis* EGU45–30 mol% with mixed bacterial culture (5MC1) (Table 1).

Influence of CG on PHA Production from Co-utilization of Biowastes

In view of the fact that OP turned out to be the best performer in terms of PHA production either individually or along with CG, we checked the influence of AP, PS and PP on this combination. Hence, OP was mixed with these wastes in different ratios.

Apple and Onion Wastes

The combination of OP: AP in different ratios produced PHA in the range of 100–250 mg/L *B. thuringiensis* EGU45. Addition of CG (1%, v/v) to OP + AP helped in enhancing PHA content i.e. up to 400 mg/L, which was 4-fold higher than the control (Table 2). Although, there was an increase in PHA production however, HV content did not improve significantly and continued to be around 75–82 mol% (Table 2). In conclusion, combination—OP: AP:: 2:1 +1% CG proved to be the most effective for PHA production by *B. thuringiensis* EGU45. Similarly, co-utilization of these wastes along with CG also proved to be

Table 2 Bacterial polyhydroxyalkanoate production by co-utilisation of biowastes and crude glycerol

Feed	Bacterial culture									
	<i>Bacillus thuringiensis</i> EGU45					Mixed culture				
	DCM (mg/L)	PHA (mg/L)	%	Mol%		DCM (mg/L)	PHA (mg/L)	%	Mol%	
HB				HV	HB				HV	
<i>OP:AP</i>										
OP:AP::1:1	2000	245	12	20	80	1260	290	23	41	58
OP:AP::1:2	2010	250	12	35	65	570	28	5	64	36
OP:AP::2:1	2200	100	4	20	80	1450	260	18	25	75
OP:AP::1:1 + 1% CG	2800	385	14	25	75	1400	280	20	23	77
OP:AP::1:2 + 1% CG	2800	350	13	25	75	1590	216	14	35	65
OP:AP::2:1 + 1% CG	3030	400	13	18	82	2400	275	11	20	80
<i>OP:PS</i>										
OP:PS::1:1	2000	185	10	35	68	1800	90	5	50	50
OP::PS::1:2	1550	125	15	80	20	2600	65	3	95	5
OP:PS::2:1	1900	80	5	65	35	2000	300	15	90	10
OP:PS::1:1 + 1% CG	2800	325	12	20	80	2300	35	12	10	90
OP:PS::1:2 + 1% CG	2700	1230	45	20	80	1600	280	10	35	65
OP:PS::2:1 + 1% CG	3700	1300	25	15	85	2600	265	5	35	65
<i>OP:PP</i>										
OP:PP::1:1	1200	40	4	75	25	950	115	12	95	5
OP:PP::1:2	1500	50	3	60	40	650	71	11	98	2
OP:PP::2:1	600	30	3	66	34	570	35	6	0	0
OP:PP::1:1 + 1% CG	900	75	8	60	40	900	87	9	97	3
OP:PP::1:2 + 1% CG	1000	20	3	75	25	975	66	7	98	2
OP:PP::2:1 + 1% CG	900	55	9	63	37	450	45	10	0	0

PS pea-shells, PP potato peels, AP apple pomace, OP onion peels, CG crude glycerol, DCM dry cell mass, PHA polyhydroxyalkanoate, HB hydroxybutyrate, HV hydroxyvalerate

effective for improving PHA yield and composition with mixed bacterial cultures (Table 2).

Pea-Shells and Onion Waste

Mixtures of OP and PS without CG in diverse combinations resulted in drastic reduction in PHA yield to 80–185 mg/L in comparison to those recorded with either OP or AP. However, addition of CG (1%, v/v) resulted in improving PHA production by *B. thuringiensis* EGU45, which was 16.2-fold at 2:1, 9.8-fold at 1:2 and 1.7-fold at 1:1 in comparison to respective controls (Table 2). Although HV content in the OP and PS combinations was lower than that recorded with AP and OP, however, addition of CG didn't result in any significant improvement. Although, with mixed microbial cultures PHA yields and their compositions from mixed biowastes (OP and PS) were lower than those recorded with *B. thuringiensis* EGU45, however, they were better than their respective controls (Table 2).

Potato and Onion Wastes

When OP and PP hydrolysates were mixed in different ratios, PHA production by *B. thuringiensis* EGU45 was quite low. In these cases PHA co-polymer content was also negligible. However, with CG (1%, v/v) as supplementation, the PHA content increased up to 75 mg/L at 1:1 of OP: PP. Addition of CG also led to reduction in HV content. Similar observations were made with mixed microbial cultures (Table 2).

In conclusion, OP turned out to be the best feed for PHA co-polymers production. Addition of CG proved effective in improving PHA and its co-polymer production with *B. thuringiensis* EGU45 and mixed microbial cultures. OP and PS mixed in 1:2 or 2:1 ratio along with CG proved effective in enhancing PHA yields.

Discussion

Different reports have shown the PHA producing ability of *Bacillus* species by co-utilizing different biowastes as feed substrates [1, 2, 6, 9, 12, 13]. Production of PHA from biowastes such as PP, OP, AP and PS has been reported with *B. thuringiensis* EGU45 and *B. cereus* EGU43 [3, 9, 14]. Subsequently, these wastes were studied in different combinations as feed for PHA production. It was realized that PS and OP hydrolysates along with glucose resulted in enhanced PHA yield and a 3HV content upto 7.5% (w/w) [3]. On the other hand, these strains have been also observed to metabolize CG, and produce PHA with a HV content of 13.4% (w/w) [10]. The potential of *B. thuringiensis* EGU45 to produce co-polymers from CG was also shown by adding propionic acid. In addition it was also observed that *B. thuringiensis* EGU45 can also tolerate a combination of CG and glucose [1]. Hence, in the present study, we have combined biowastes and CG to explore the possibility of extending the metabolic limits of *B. thuringiensis* EGU45 to produce PHA and its co-polymers. It was observed that PS and OP hydrolysates along with CG were the best combinations for PHA production by *B. thuringiensis* EGU45, as was the case where glucose was used as supplement [3]. The unique feature of the present study is that OP:AP::2:1 along with 1% v/v, CG could produce 1300 mg/L of PHA with a enhanced HV content of 21.2% (w/w). In addition, here the need for supplementation with precursors [1] for PHA co-polymers was replaced with naturally occurring biowastes. These precursors are generally volatile fatty acids (VFAs) such as propionic acid (PA), butyric acid (BA), acetic acid (AA) and iso-valeric acid (IVA), which act as intermediates in PHA co-polymer production [3, 4, 8, 9]. Here, this enhancement in PHA content was seen with change in metabolism i.e. addition of CG to biowastes. This led to changes in PHA co-polymer compositions (3HB:3HV mol%)—55:45, 85:15, 81:19, 28:72 by *B. thuringiensis* EGU45 and 5MC1 (Tables 1, 2).

Copolymers of PHA have gained attention due to their various biomedical applications. Due to their lower crystallinity, P (3HB-4HB) were employed for drug delivery and making medical devices such as: rivets, tacks, pins, sutured fastener etc. [15]. PHA co-polymers produced by *Pseudomonas fluorescens* act as immobilizing agent in clinical diagnostics [16]. *Aeromonas hydrophila* produces PHA copolymers poly-3-hydroxybutyrate-hydroxyhexanoate (PHB-HHx), which are useful in preparing vessel stent [15]. Poly-3-hydroxy-actylthioalkanoate-co-3-hydroxyalkanoate used as antibacterial agent against *Staphylococcus aureus* [17, 18]. PHB-HHx has been exploited for osteoblast attachment, proliferation and differentiation

[19, 20]. PHA-copolymers can be used as scaffolds for liver tissue engineering, cartilage repair and promoting cell growth [21–23].

In general, we expect different wastes to get mixed while transportation hence, we need to treat wastes in combinations. The need is to have a versatile organism or a combination of organisms, which can withstand physiological stress arising out of variation in feed material [9, 10]. Since, *Bacillus* has a unique ability to produce hydrogen and PHA from different wastes even in non-limiting nitrogen conditions [10, 24], hence, this study will help in further exploiting them on different feed material in diverse combinations. *Bacillus* is an organism which is generally regarded as safe (GRAS). Hence, it can be used in an unrestricted manner without harming environment [4, 25, 26].

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Compliance with ethical standards

Conflict of interest None.

References

1. Ray S, Kalia VC (2017) Co-polymers of substrates by *Bacillus thuringiensis* regulates polyhydroxyalkanoates co-polymer compositions. *Bioresour Technol* 224:743–747. <https://doi.org/10.1016/j.biortech.2016.11.089>
2. Ray S, Kalia VC (2017) Microbial cometabolism and polyhydroxyalkanoate co-polymers. *Indian J Microbiol* 57:39–47. <https://doi.org/10.1007/s12088-016-0622-4>
3. Kumar P, Ray S, Kalia VC (2016) Production of co-polymers of polyhydroxyalkanoates by regulating the hydrolysis of biowastes. *Bioresour Technol* 200:413–419. <https://doi.org/10.1016/j.biortech.2015.10.045>
4. Singh M, Kalia VC (2015) Challenges and opportunities for customizing polyhydroxyalkanoates. *Indian J Microbiol* 55:235–249. <https://doi.org/10.1007/s12088-015-0528-6>
5. Opgenorth PH, Korman TP, Bowie JU (2016) A synthetic biochemistry module for production of bio-based chemicals from glucose. *Nat Chem Biol* 12:393–395. <https://doi.org/10.1038/nchembio.2062>
6. Albuquerque MGE, Martino V, Pollet E, Averous L, Reis MAM (2011) Mixed culture polyhydroxyalkanoate (PHA) production from volatile fatty acid (VFA)-rich streams: effect of substrate composition and feeding regime on PHA productivity, composition and properties. *J Biotechnol* 151:66–76. <https://doi.org/10.1016/j.jbiotec.2010.10.070>
7. Patel SKS, Singh M, Kumar P, Purohit HJ, Kalia VC (2012) Exploitation of defined bacterial cultures for production of hydrogen and polyhydroxybutyrate from pea-shells. *Biomass Bioenergy* 36:218–225. <https://doi.org/10.1016/j.biombioe.2011.10.027>

8. Singh M, Kumar P, Patel SKS, Kalia VC (2013) Production of polyhydroxyalkanoate co-polymer by *Bacillus thuringiensis*. Indian J Microbiol 53:77–83. <https://doi.org/10.1007/s12088-012-0294-7>
9. Kumar P, Singh M, Mehariya S, Patel SKS, Lee JK, Kalia VC (2014) Ecobiotechnological approach for exploiting the abilities of *Bacillus* to produce co-polymer of polyhydroxyalkanoate. Indian J Microbiol 54:151–157. <https://doi.org/10.1007/s12088-014-0457-9>
10. Kumar P, Ray S, Patel SK, Lee JK, Kalia VC (2015) Bioconversion of crude glycerol to polyhydroxyalkanoate by *Bacillus thuringiensis* under non-limiting nitrogen conditions. Int J Biol Macromol 78:9–16. <https://doi.org/10.1016/j.ijbiomac.2015.03.046>
11. Kalia VC, Prakash J, Koul S (2016) Biorefinery for glycerol rich biodiesel industry waste. Indian J Microbiol 56:113–125. <https://doi.org/10.1007/s12088-016-0583-7>
12. Anil-Kumar PK, Shamla TR, Kshama L, Prakash MH, Joshi GJ, Chandrashekar A, Kumari KSL, Divyashree MS (2007) Bacterial synthesis of poly(hydroxybutyrate-co-hydroxyvalerate) using carbohydrate-rich mahua (*Madhuca* sp.) flowers. J Appl Microbiol 103:204–209. <https://doi.org/10.1111/j.1365-2672.2006.03221.x>
13. Bengtsson S, Werker A, Christensson M, Welander T (2009) Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. Bioresour Technol 99:509–516. <https://doi.org/10.1016/j.biortech.2007.01.020>
14. Kumar T, Singh M, Purohit HJ, Kalia VC (2009) Potential of *Bacillus* sp. to produce polyhydroxybutyrate from biowaste. J Appl Microbiol 106:2017–2023. <https://doi.org/10.1111/j.1365-2672.2009.04160.x>
15. Ray S, Kalia VC (2017) Biomedical applications of polyhydroxyalkanoates. Indian J Microbiol 57:261–269. <https://doi.org/10.1007/s12088-017-0651-7>
16. Ihssen J, Magnani D, Thony-Meyer L, Ren Q (2009) Use of extracellular medium chain length polyhydroxyalkanoate depolymerase for targeted binding of proteins to artificial poly [(3-hydroxyoctanoate)-co-(3-hydroxyhexanoate)] granules. Biomacromol 10:1854–1864. <https://doi.org/10.1021/bm9002859>
17. Dinjaski N, Fernandez-Gutierrez M, Selvam S, Parra-Ruiz FJ, Lehman SM, San Roman J, Garcia E, Garcia JL, Garcia AJ, Prieto MA (2014) PHACOS, a functionalized bacterial polyester with bactericidal activity against methicillin-resistant *Staphylococcus aureus*. Biomaterials 35:14–24. <https://doi.org/10.1016/j.biomaterials.2013.09.059>
18. Martinez V, Dinjaski N, De Eugenio LI, De la Pena F, Prieto MA (2014) Cell system engineering to produce extracellular polyhydroxyalkanoate depolymerase with targeted applications. Int J Biol Macromol 71:28–33. <https://doi.org/10.1016/j.ijbiomac.2014.04.013>
19. Wang YW, Wu Q, Chen J, Chen GQ (2005) Evaluation of three-dimensional scaffolds made of blends of hydroxyapatite and poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) for bone reconstruction. Biomaterials 26:899–904. <https://doi.org/10.1016/j.biomaterials.2004.03.035>
20. Wang Y, Bian YZ, Wu Q, Chen GQ (2008) Evaluation of three-dimensional scaffolds prepared from poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) for growth of allogeneic chondrocytes for cartilage repair in rabbits. Biomaterials 29:2858–2868. <https://doi.org/10.1016/j.biomaterials.2008.03.021>
21. Chen W, Tong YW (2012) PHBV microspheres as neural tissue engineering scaffold support neuronal cell growth and axon-dendrite polarization. Acta Biomater 8:540–548. <https://doi.org/10.1016/j.actbio.2011.09.026>
22. Canadas RF, Cavalheiro JMBT, Guerreiro JDT, de Almeida MCMD, Pollet E, da Silva CL, da Fonseca MMR, Ferreira FC (2014) Polyhydroxyalkanoates: waste glycerol upgrade into electrospun fibrous scaffolds for stem cells culture. Int J Biol Macromol 71:131–140. <https://doi.org/10.1177/0885328216639749>
23. Ching KY, Andriotis OG, Li S, Basnett P, Su B, Roy I, Stolz M (2016) Nanofibrous poly (3-hydroxybutyrate)/poly (3-hydroxyoctanoate) scaffolds provide a functional microenvironment for cartilage repair. J Biomater Appl 31:77–91. <https://doi.org/10.1177/0885328216639749>
24. Patel SKS, Lee JK, Kalia VC (2017) Dark-fermentative biological hydrogen production from mixed biowastes using defined mixed cultures. Indian J Microbiol 57:171–176. <https://doi.org/10.1007/s12088-017-0643-7>
25. Singh M, Patel SKS, Kalia VC (2009) *Bacillus subtilis* as potential producer for polyhydroxyalkanoates. Microb Cell Fact 8:38. <https://doi.org/10.1186/1475-2859-8-38>
26. Kumar P, Patel SKS, Lee JK, Kalia VC (2013) Extending the limits of *Bacillus* for novel biotechnological applications. Biotechnol Adv 31:1543–1561. <https://doi.org/10.1016/j.biotechadv.2013.08.007>