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Production and Characterization of Polyhydroxybutryrate Biopolymer from *Azohydromonas australica* Using Sucrose as a Sole Carbon Source

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Abstract

A bacterial strain *Azohydromonas australica* DSM 1124 has been chosen, which is accumulated intracellular poly-β-hydroxybutyrate particles, each method which has been merit and demerit for employed in PHB extraction. Our study, we have selected chloroform-sodium hypochlorite method. It is one of the methods for extracting PHB forms *Azohydromonas australica* DSM 1124. Polyhydroxybutyrate is a biodegradable and biocompatible thermoplastic with many interesting applications in medicine, food packaging and tissue engineering materials. That does not produce any toxins or residues in the environment like petroleum based plastics. The present study was emphasized on enhanced production of PHB by *Azohydromonas australica* using sucrose as a sole carbon source and estimation of biomass and sucrose content in the media.The batch kinetics analysis of *Azohydromonas australica* was done an interval of 3 h. Batch cultivation with optimized media recipe in a 7 L bioreactor exhibited a maximum biomass 1.71 g/L and PHB concentration 2.67 g/L for *A.* australica. The characterization of PHB was done by growth kinetics studies, UV-Spectrophotometer and Fourier transforms infrared spectroscopy (FTIR).

Keywords: Polyhydroxybutyrate; *Azohydromonas australica*; Sucrose; FT-IR

Introduction

Biopolymers are polymers that are generated from renewable natural sources, are often biodegradable and nontoxic. They can be produced by biological systems (like, microorganisms, plants and animals) or chemically synthesized from biological materials (like, sugars, starch, natural fats and oils). There are two strategies applied for converting these raw materials into biodegradable polymers: extraction of the native polymer from a plant or animal tissue and a chemical or biotechnological route of monomer polymerization. Biodegradable biopolymers (BDP) are an alternative way for the production of petroleum-based polymers like, traditional plastics. Some BDP degrade in only a few weeks, while the others take several months for the degradation. In principle, the properties relevant for application as well as biodegradability are determined on the basic of the molecular structure and characterization. According to the *American Society for Testing and Materials*, biopolymers are degradable in results from the action of naturally occurring microorganisms such as bacteria, fungi and algae [1]. The BDP can be produced from natural occurring raw materials such as starch, sugar, cellulose as well as fossil oils. Besides the BDP biodegradability, other relevant aspects for processing are also important including, thermal stability and viscosity that are used as a conventional technology without any large adaptations. This economic value of renewable raw materials will increase in a significant extent and stimulate new industrial activities [2,3]. The potential for biodegradable of polymers are depends on legislation, local directives and sufficient waste treatment capacity at composting plants (for packaging, disposables, foils for agricultural use). Similarly, the development in the chemical sector (like, solvents, glues, coatings) are also become an important encouragement for the application of degradable raw materials. A new biotechnological process have direct implications for society but there are significant ethical issues publicly and personally concern to sustain for the social development [4,5]. To overcome of these issues, they can widely use of BDP to prevent or reduce toxic effect of industrial waste in the environment and providing a high level of environmental protection. The BDP also protects the loss of soil fertility and significantly enhance soil carbon level.

Biodegradable plastics are either partly or fully degraded either by non-enzymatic or enzymes activity possessed by some microorganisms that lead to a chain breakage of the polymer into monomers. Some type of the biodegradable plastics being produced polyhydroxyalkanoates (PHAs), polylactides, aliphatic polyesters, polysaccharides, and copolymers and blends of starch and polypropylene [6,7]. Blends of starch and polypropylene can be regarded only as semi-biodegradable while PHA is 100% biodegradable [8]. One of most widely used biodegradable plastics is polylactic acid (PLA) for petroleum derived plastics but it is costly than polyhydroxybutyrate (PHB) [9]. But there is several demerits in PLA including, slow crystallization, poor heat resistance and mechanical brittleness [10]. In addition, although lactic acid can currently be produced by industrial scale fermentation, it still needs chemical polymerization to get PLA, which makes the production process more complicated and time-consuming [9]. Hence, further exploration of other biodegradable plastics like PHB is warranted. PHB belongs to the class of biodegradable plastics, polyhydroxyalkanoates (PHAs).

PHB is a natural biopolymers, that are synthesized and catabolized by microorganisms particularly bacteria [11,12]. There are more than 300 of such species known to produce PHB's [13]. A number of bacteria such as Azotobacter, Bacillus, *Azohydromonas australica,*

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Archaebacteria, Methylobacteria and Pseudomonas have been found to accumulate PHB to varying levels. *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*) was found to accumulate PHBs up to 80 percent dry weight [14]. Macrae and Wilkinson [15] reported that Properties of biodegradability of PHB produced by *B. cereus* and *B. megaterium*. The shape, size, structure and physical properties of these species differ from organism to organism [16]. Beijerinck was the first to observe lucent granules of PHB in bacterial cells in 1888 [17]. Hence, in the previous studies showed that *Bacillus cereus, Bacillus subtillis*, *B. megaterium* have been shown to produce higher amounts of PHB using agro-industrial waste with variable level of carbon sources [18]. Besides recent studies revealed that the highest production of PHB using *Bacillus thuringiensis* along with H_2 gas, glucose: $NaNO_3$ in 4:1 ratio [19,20]. PHB are comparable to commonly used in bulk plastics like, polypropylene. It is a unique natural biopolymer, which have three significant features like, thermoplastic processability, 100% water and moisture resistance and 100% biodegradability [21]. It could therefore be used for applications similar to those of common plastics and would fit well into new waste-management strategies. PHB also has some addition advantages that monomer 3-hydroxybutyric acid is a chiral molecule. These monomer units of PHB can be used as basic molecules for the production in pharmaceutical or agro-chemical agents [22]. PHB is biocompatible and nontoxic in nature. The rate of PHB degradation is very slow *in vivo* and rate limiting release of drugs [23]. The aim of our study is to evaluate the biopolymer PHB producing *Azohydromonas australica* DSM 1124 from German collection of microorganisms and cell cultures (DSMZ, Germany) was obtained as a gift strain from IIT Delhi. The isolated bacterial cells were identified and characterized by morphological, biochemical, physiological and molecular characteristics. The PHB was extracted and purified after mass culture of the selected bacterial strain *Azohydromonas australica*. The purified PHB was then identified and characterized by various analytical methods such as growth kinetics study, Ultraviolet-visible Spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR).

Materials and Methods

Micro-organism and maintenance

Azohydromonas australica DSM 1124 from German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) was obtained as a gift strain from IIT Delhi. The strain was maintained on nutrient agar (Hi-Media, India) slants at 4°C and sub-cultured monthly.

Growth kinetics studies

Media composition for growth kinetics study of *Azohydromonas australica* was prepared containing : sucrose 25 g/L, $(NH_4)(NO_3)$ 2.8 g/L, KH_2PO_4 3.25 g/L, K_2HPO_4 3.25 g/L, $MgSO_4$ 0.2 g/L (Himedia) and trace element solution 1.5 mL/L. KH_2PO_4 and $MgSO_4$ were sterilized separately to prevent precipitation and added to the medium in laminar air flow. TES was filter sterilized and added to the medium in sterile environment. The parent culture of *A. australica* was inoculated (5%) in 100 ml media in different flasks. The flasks were kept in an incubator shaker at 33°C and 200 rpm. Temperature and pH was maintained at 33°C and 7, respectively. Samples were taken at intervals of 3 h for the purpose of taking optical density. Wet weight of pellets was taken to estimate biomass of organism.

Extraction of polyhydroxybutyrate (PHB) and quantification

The extraction of polyhydroxybutyrate (PHB) from *Azohydromonas australica* was described in detail by Xu and Chen [24]. Briefly, cultures were incubated for 48 h at the optimal growth temperature. Bacterial cells were centrifuged at 6000 rpm for 15 min and were dried at room temperature for 24 h. The dry weight of the pellets was determined. Bacterial cell walls were lysed by adding sodium hypochloride, mixing and incubating at 37°C for 1 h. Supernatant was obtained by centrifugation and transferred to a Soxhlet system. Cell lipids and other molecules were treated with 5 ml of 96% ethanol and acetone. PHB was extracted by hot chloroform (adding 10 ml chloroform in a water bath). Then chloroform was evaporated to obtain PHB crystals. By adding 10 ml of 98% sulfuric acid at 60°C for 1 h in water bath after this PHB crystals were converted into crotonic acid. The absorbance of the solution was measured at 235 nm in a UV-visible spectrophotometer against a sulfuric acid used as a blank. The amount of PHB per gram dry weight of bacterial cells was determined using a standard curve of PHB.

Characterization of polyhydroxybutyrate

The polyhydroxybutyrate (PHB) was further characterized by UV-visible Spectrophotometer and Fourier Transform Infrared Spectroscopy (FTIR).

UV-visible spectrophotometer

Absorbance spectra of PHB isolated from *Azohydromonas Australica* was measured at 235 nm using sulphuric acid as a blank under UV-visible Spectrophotometer (Shimadzu, model UV 1700).

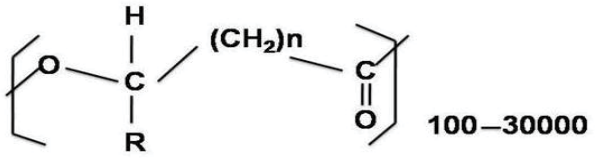
Fourier transforms infrared spectroscopy (FTIR) studies

For FTIR analyses, the PHB samples were dissolved in chloroform and then added to KBr pellets. After complete solvent evaporation, FTIR spectra were recorded using a spectrometer (Shimadzu FTIR 8201-PC). A total of 20 scans were recorded at a 2 cm^{-1} resolution, between 4000 and 1000 cm^{-1}.

Results and Discussion

Extraction of polyhydroxybutarate (PHB)

There are no reports on extraction of PHB from *Azohydromonas australica* spp. In our studies, we have performed only one method to extract PHB from *Azohydromonas australica* DSM 1124. Chloroform-sodium hypochlorite method is one of the PHB extractions from *Azohydromonas australica* DSM 1124, which has provided a 73% extraction rate with more than 90% purity and nontoxic for the macromolecules. After treated by sodium hypochlorite, cells break up and PHB disperse in solution. PHB were rapidly transferred to the chloroform phase, which accelerate the extraction and shorten the contact time of PHB with the sodium hypochlorite. Another biological method for the PHB production by recombinant *Bacillus subtilis* PT5(ReA)/pBmRBC was induced by IPTG. The effect of IPTG concentration and cell density on PHB yield was evaluated. PHB content increased as a function of IPTG dosage. *B. subtilis* is a GRAS organism that has many advantages for the large-scale production of chemicals, enzymes, and amino acids, including the fact that it does not produce enzymes that degrade PHA. We therefore examined in the present study whether *B. subtilis* can be an effective vehicle for PHB production (Figure 1). The PHB synthesis genes and mechanisms of *B. megaterium* and *R. eutropha* have been well characterized and served as a basis for creating a recombinant *B. subtilis* strain that can synthesize PHB than other carbon sources (Table 1). A comparative chart between the productions of PHB using *Alcaligenes eutrophus vs. Azohydromonas australica* as summarized in Table 1.



n=1 R= hydrogen Poly(3-hydroxypropoinate)
R= methyl Poly(3-hydroxybutarate)
R= ethyl Poly(3-hydroxyvalerate)
R= propyl Poly(3-hydroxyhexanoate)
R= pentyl Poly(3-hydroxyoctanoate)
R= nonyl Poly(3-hydroxydodecanoate)

n=2 R= hydrogen Poly(4-hydroxybutarate)
R= methyl Poly(4-hydroxyvalerate)

n=3 R= hydrogen Poly(5-hydroxyvalerate)
R= methyl Poly(5-hydroxyhexanoate)

n=4 R= hexyl Poly(6-hydroxydodecanoate)

Figure 1: General structure of Polyhydroxyalkanoates (PHAs).

S. No.	*Alcaligenes eutrophus*	*Azohydromonas australica*
1	Glucose as a carbon source	Sucrose as a carbon source
2	PHB production equal to 80% of their dry body weight	PHB production more than 80% of their dry body weight
3	PHB obtained 15 g/L	PHB obtained 3.17 g/L
4	PHB contains 5.3%	PHB contains 29%
5	Biomass contains 36%	Biomass contains 37%
6	Maximum specific growth rate 0.075 to 0.300 h^{-1}	Maximum specific growth rate 0.26 h^{-1}

Table 1: Comparative chart between the productions of PHB using *Alcaligenes eutrophus vs. Azohydromonas australica.*

Batch kinetics profile of *Azohydromonas australica*

Kinetic analysis of *Azohydromonas australica* was performed after an interval of 3 h. Biomass and sucrose content in the media was estimated by wet weight basis. Sucrose content was estimated by dinitrosalisylic (DNS) method. It was clear from the data that the exponential phase of *A. australica* begins from 9 h till 36 h after that decline phase started. The mmaximum biomass of 1.17 g/L was observed at 36 h and after that growth curve was declined (Figures 2 and 3 and Tables 2 and 3). Similarly, sucrose content showed significantly decreases with time as summarized in Table 2 and Figure 2.

Polyhydroxybutyrate (PHB) quantification

The estimation of PHB isolated from *A. australica* was calculated by UV-visible spectrophotometer at 235 nm as summarized in Table 3 and Figure 3. It is clear from the data that the maximum PHB concentration reaches to 2.67 g/L in 36 h (Table 3).

Characterization of the intracellular PHB

The purified PHB was characterized by UV-Spectrophotometer analysis and Fourier Transform Infrared Spectroscopy (FTIR).

Ultraviolet absorption spectroscopy

When PHB dissolved in sulphuric acid, it converted into crotonic acid, which has a maximal absorption at 235 nm. The UV spectra of samples isolated from *Azohydromonas australica* DSM 1124 that of the

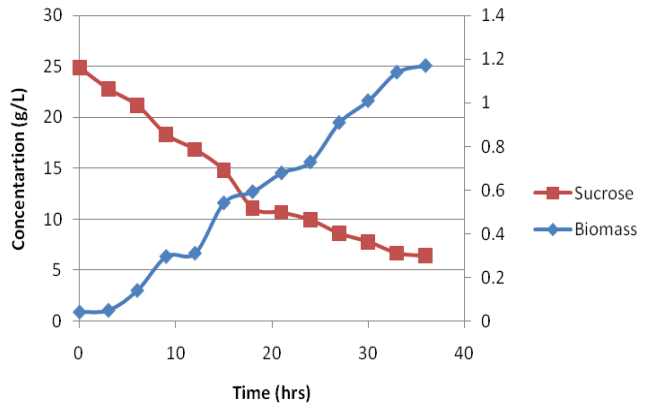


Figure 2: Batch kinetics profile of *A. australica.*

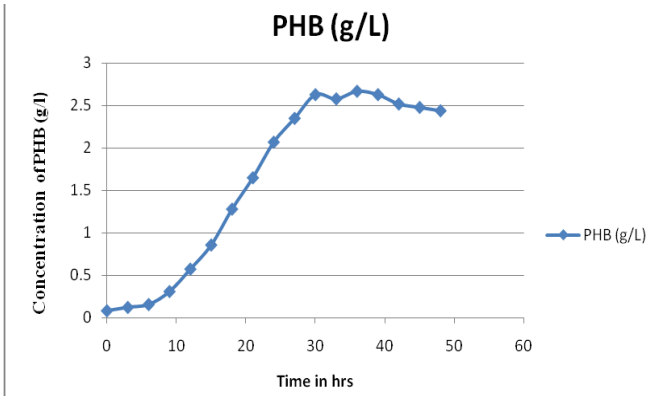


Figure 3: Batch production profile of *A. Australica* for PHB production.

S. No.	Time	Biomass g/L	Sucrose g/L
1	0 h	0.044	24.856
2	3 h	0.052	22.764
3	6 h	0.143	21.168
4	9 h	0.298	18.309
5	12 h	0.313	16.837
6	15 h	0.543	14.778
7	18 h	0.594	11.091
8	21 h	0.68	10.665
9	24 h	0.73	9.932
10	27 h	0.91	8.605
11	30 h	1.01	7.803
12	33 h	1.14	6.687
13	36 h	1.17	6.434
14	39 h	1.03	6.312
15	42 h	0.98	5.567
16	45 h	0.95	5.333
17	48 h	0.84	5.189

Table 2: Batch kinetics of *A. australica* for biomass and sucrose concentration.

standard completely. According to the peak value, the content of PHB can be calculated by a PHB standard curve (data not shown) [25-28]. Concentrated H_2SO_4 destroys cell membrane, and the fragments of cell have no absorption at 235 nm. Therefore, the quantity of PHB in ferment medium is calculated by digesting the cells directly.

S. No.	Time	PHB g/L
1	0 h	0.081
2	3 h	0.121
3	6 h	0.155
4	9 h	0.308
5	12 h	0.573
6	15 h	0.8564
7	18 h	1.28
8	21 h	1.65
9	24 h	2.07
10	27 h	2.35
11	30 h	2.63
12	33 h	2.58
13	36 h	2.67
14	39 h	2.63
15	42 h	2.52
16	45 h	2.48
17	48 h	2.44

Table 3: Summarized batch production data of *A. australica* for PHB production.

Fourier transforms infrared (FT-IR) spectroscopy

FTIR data of PBS isolated from *Azohydromonas australica* DSM 1124 reveals the presence of electron-dense spherical granules (Figure 4). The FTIR spectra showed the practically identical structure of the standard PHB and the biopolymers produced by *Azohydromonas australica* DSM 1124 (Figure 4) [29]. Characteristic bands for PHB were obtained for the samples. The band found at d 1460 cm^{-1} corresponds to the asymmetrical deformation of the C–H bond in CH_2 groups, while the one found at 1380 cm^{-1} is the equivalent for CH_3 groups. The bands found at 1721 cm^{-1} correspond to the stretching of the C=O bond, whereas a series of intense bands located at 1017–1074 cm^{-1} correspond to the stretching of the C–O bond of the ester group [30-33]. Also bands of minor relevance, such as those found at 3481 cm^{-1}, originated by terminal OH groups or to water adsorption onto the sample, are found in all spectra. The analyzed results correlated with the report of confirming that the isolated compound was PHB [34] (Figure 4).

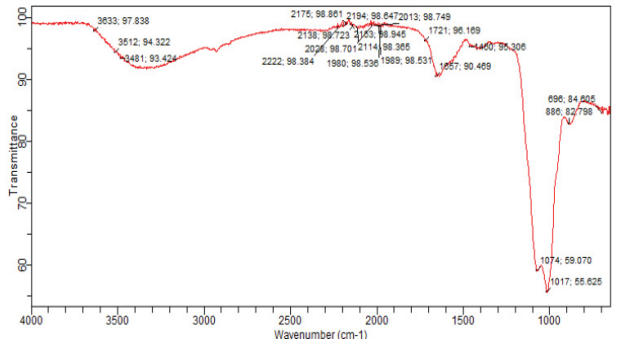


Figure 4: Fourier transform infrared spectroscopy (FTIR) of biopolymer PHB from *Azohyromonas australica* DSM 1124.

Conclusion

The present study demonstrated that optimization of fermentation medium recipe by statistical means play critical role in enhancing the biomass and PHB concentration with a minimum number of experiments. Statistically optimized media recipe for cell growth and PHB formation was as follows: sucrose 25 g/L, $(NH_4)(NO_3)$ 2.8 g/L, KH_2PO_4 3.25 g/L, K_2HPO_4 3.25 g/L, $MgSO_4$ 0.2 g/L and trace element solution 1.5 mL/L. Batch cultivation with optimized media recipe in a 7 L bioreactor exhibited a maximum biomass 1.71 g/L and PHB concentration 2.67 g/L for *A. australica*. The main challenge of our studied to industrialize PHB production on the expense of raw materials which amounts to around 40% of the total cost [35]. Therefore, looking for cheaper feed stocks should be the first research direction towards the commercialized of PHB production using biomass. Another issue relates to the drawbacks of PHB like its brittleness and thermal instability which greatly narrow its application. Therefore, the next step towards enhancing the applicability of biodegradable polymers is to explore the production of copolymers, which have improved mechanical and thermal properties. For example, the incorporation of 3HV monomers to make PHBHV lowers the melting point and crystallinity of PHB, thus deceasing stiffness and increasing toughness. This makes it easier to process and acquire more favorable properties for commercial application [36]. However, the production of PHB through transgenic plants, which are capable of producing larger amounts of PHB within the same time. The production of PHB in plants could allow synthesis of up to million ton compared to thousand ton scale which fermentation production can provide [36]. However, key barriers to be overcome for plant-based PHB production include low PHB content, more complex product recovery and downstream processing due to interference by other plant components, as well as severely stunted plant growth by the PHB produced [37]. In conclusion, whatever the subsequent research area, one important thing to be considered is to build a comprehensive economic analysis model for the production of PHB from renewable biomass sources. A balance between operating cost, product yield and quality is imminent to make this conversion more economically and functionally feasible.

References

1. Shimao M (2001) Biodegradation of plastics. Curr Opin Biotechnol 12: 242-247.
2. Landsbergis PA, Cahill J, Schnall P (1999) The impact of lean production and related new systems of work organization on worker health. J Occup Health Psychol 4: 108-130.
3. Van den Bosch CJH, Weterings R.A.P.M (1997) Methods for the calculation of physical effects: Due to releases of hazardous materials (liquids and Gases),'yellow Book'. CPR 14E [in Two Parts]. Sdu Uitgevers.
4. Harding KG, Dennis JS, Von Blottnitz H, Harrison STL (2007) Environmental analysis of plastic production processes: Comparing petroleum-based polypropylene and polyethylene with biologically-based poly-ß-hydroxybutyric acid using life cycle analysis. J Biotechnol 130: 57-66.
5. Poirier Y, Nawrath C, Somerville C (1995) Production of polyhydroxyalkanoates, a family of biodegradable plastics and elastomers, in bacteria and plants. Biotechnology (N Y) 13: 142-150.
6. Lee SY (1996) Bacterial polyhydroxyalkanoates. Biotechnol Bioeng 49: 1-14.
7. Patwardhan PR, Srivastava AK (2004) Model-based fed-batch cultivation of *R. eutropha* for enhanced biopolymer production. Biochem Engg J 20: 21-28.
8. Datta A, Baird DG (1995) Compatibilization of thermoplastic composites based on blends of polypropylene with two liquid crystalline polymers. Polymer 36: 505-514.
9. Steinbüchel A (1991) Polyhydroxyalkanoic acids. In Biomaterials Palgrave Macmillan UK, pp: 123-213.
10. Yokohara T, Yamaguchi M (2008) Structure and properties for biomass-based polyester blends of PLA and PBS. Eur Polym J 44: 677-685.
11. Akar A, Akkaya EU, Yesiladali SK, Celikyilmaz G, Cokqor EU, et al. (2006) Accumulation of polyhydroxyalkanoates by *Microlunatus phosphovorus* under various growth conditions. J Ind Microbiol Biotechnol 33: 215-220.
12. Berlanga M, Montero MT, Hernández-Borrell J, Guerrero R (2010) Rapid spectrofluorometric screening of poly-hydroxyalkanoate-producing bacteria from microbial mats. Int Microbiol 9: 95-102.

13. Hrabak O (1992) Industrial production of poly-ß-hydroxybutyrate. FEMS Microbiol Lett 103: 251-255.

14. Lee SY (1996) Plastic bacteria-progress and prospects for polyhydroxyalkanoate production in bacteria. Trends Biotechnol 14: 431–438.

15. Macrae RM, Wilkinson JF (1958) Poly-beta-hyroxybutyrate metabolism in washed suspensions of *Bacillus cereus* and *Bacillus megaterium*. J Gen Microbiol 19: 210-222.

16. Ha CS, Cho WJ (2002) Miscibility, properties, and biodegradability of microbial polyester containing blend. Prog Polym Sci 27: 759-809.

17. Chowdhury AA (1963) Poly-beta-hydroxybutyric acid-splitting bacteria and an exoenzyme. Arch Microbiol 47: 167-200.

18. Ghate B, Pandi P, Kulkarni C, Mungi DD, Patel TS (2011) PHB Production using novel agro-industrial sources from different Bacillus species. Int J Pharm Biol Sci 2: 242-249.

19. Singh M, Kumar P, Patel SK, Kalia VC (2013) Production of polyhydroxyalkanoate co-polymer by *Bacillus thuringiensis*. Indian J Microbiol 53: 77-83.

20. Pal A, Prabhu A, Kumar AA, Rajagopal B, Dadhe K, et al. (2009) Optimization of process parameters for maximum poly(-beta-)hydroxybutyrate (PHB) production by *Bacillus thuringiensis* IAM 12077. Pol J Microbiol 58: 149-154.

21. Hrabak O (1992) Industrial production of poly-ß-hydroxybutyrate. FEMS Microbiol Lett 103: 251-255.

22. Seebach D, Roggo S, Zimmermann J (1987) Biological-chemical preparation of 3-hydroxycarboxylic acids and their use in EPC-synthesis. In: Stereochemistry of Organic and Bioorganic Transformations (Bartmann, W. And K.B. Sharpless, Eds.). VCH Verlagsgesellschaft, Weinheim, pp: 85-126.

23. Korsatko W, Wabnegg B, Tillian HM, Braunegg G, Lafferty RM (1983) Poly-d-(-)-3-hydroxybutyric acid-a biodegradable carrier for long-term medication dosage. 2. The biodegradation in animal organism and *in vitro-in vivo* correlation of the liberation of pharmaceuticals from parenteral matrix retard tablets. Pharmazeutische Industrie 45: 1004-1007.

24. Xu XP, Chen JF (2006) Study on poly-ß-hydroxybutyrate (PHB) extraction of *Sphaerotilus natans*. Biotechnol Bull 3: 68-72.

25. Xu A, Zhang S (2008) The optimization of culture condition for PHB accumulating by *Acidiphilium cryptum* DX1-1. Wuhan Univ. Nat Sci Ed 54: 702-712.

26. Khan MA, Alam K, Dixit K, Rizvi MMA (2016) Role of peroxynitrite induced structural changes on H2B histone by physicochemical method. Int J Biol Macromol 82: 31–38.

27. Khan MA, Dixit K, Moinuddin, Arif Z, Alam K (2014) Studies on peroxynitrite-modified H1 histone: Implications in systemic lupus erythematosus. Biochimie 97: 104-113.

28. Khan MA, Dixit K, Jabeen S, Moinuddin, Alam K (2009) Impact of peroxynitrite modification on structure and immunogenicity of H2A histone. Scand J Immunol 69: 99-109.

29. Ailing XU, Yanyan LAO, Qing Zhang, Jialuo LI, Jinlan XIA (2010). Extraction and characterization of PHB from *Acidiphilium cryptum* DX1-1.

30. Rojas DGB, Manosalva JL, Liendo G (2000) Caracterización a partir de la microscopía óptica de luzpolarizada, delas propiedades térmicasyla espectrocopí eltermoplástico biodegradable poli(hidroxibutirato) Rev. Latinoam Metal Mater 20: 47-53.

31. Dixit K, Khan MA, Sharma YD, Moinuddin, Alam K (2010) Physicochemical studies on peroxynitrite-modified H3 histone. Int J Biol Macromol 46: 20-26.

32. Khan MA, Zafaryab M, Mehdi SH, Quadri J, Rizvi MMA (2017) Characterization and carboplatin loaded chitosan nanoparticles for the chemotherapy against breast cancer *in vitro* studies. Intl J Biol Macromol 97: 115-122.

33. Khan MA, Zafaryab M, Mehdi SH, Ahmad I, Rizvi MMA (2016) Characterization and anti-proliferative activity of curcumin loaded chitosan nanoparticles in cervical cancer. Intl J Biol Macromol 93: 242-253.

34. Arun A, Arthi R, Shanmugabalaji V, Eyini M (2009) Microbial production of poly-beta-hydroxybutyrate by marine microbes isolated from various marine environments. Bioresource Technol 100: 2320-2323.

35. Choi J, Lee SY (1999) Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. App Microbiol Biotech 51: 13-21.

36. Poirier Y, Nawrath C, Somerville C (1995) Production of polyhydroxyalkanoates, a family of biodegradable plastics and elastomers, in bacteria and plants. Biotechnology (N Y) 13: 142-150.

37. Nawrath C, Poirier Y, Somerville C (1994) Targeting of the polyhydroxybutyrate biosynthetic pathway to the plastids of *Arabidopsis thaliana* results in high levels of polymer accumulation. Proc Natl Acad Sci 91: 12760-12764.