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Biosynthesis of Polyhydroxybutyrate from Giant Reed Grass Hydrolysate and Evaluation of its Drug Releasing Profiles

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ABSTRACT

The cost of biopolymer production is one of the most important factor restricting the industrial application of Polyhydroxybutyrate (PHB). The main aim of this present study is to explore suitable low-cost novel substrate for PHB production. In this study, giant reed grass (GRG) biomass was pretreated with different concentration of NaOH (1-3%) at various conditions like room temperature, microwave irradiation high temperature and pressure (HTP). Among these three conditions, at the HTP pretreatment with 2% NaOH, the maximum fermentable glucose of 79.32% was obtained after removal of llignin by SC/AA treatment. Further, this pretreatment condition was used for hydrolysate preparation for PHB biosynthesis using *B. subtilis* RNM. Under optimized condition, the maximum PHB of 46.21±0.2 gL^{-1} was obtained from GRG hydrolysate. This result indicates that the GRG hydrolysate could be a novel low-cost substrate for PHB biosynthesis. This is the first attempt we made to use GRG hydrolysate as feedstock for PHB biosynthesis. Moreover, the produced PHB was characterized by ^{1}H NMR, FTIR and thermal amalysis (DSC and TGA) techniques. The characterized results were very similar to characters of commercial PHB. Further, the produced PHB was used for doxorubicin - PHB microparticles preparation with 93.21±0.15 % of encapsulation efficiency and found its anticancer drug delivery efficiency as 98%. Hence, this study demonstrates that the PHB biosynthesized from GRG hydrolysate could be a best biodegradable polymer for anticancer drug doxorubicin encapsulation and delivery.

Keywords: *B. subtilis,* Biosynthesis, giant reed grass, hydrolysate, microparticles, drug releasing, PHB

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INTRODUCTION

In recent years, disposal of synthetic plastic waste is a big ecotechnological problem. This could be solved by the development of biodegradable plastics using principles of green chemistry. Green chemistry is playing an important role in the development of eco-friendly materials for the next generation. One of such development is producing biodegradable polymers from renewable resources, which does not compete with food crops. Polyhydroxyalkanoates (PHAs) is a biodegradable linear polyesteric biological macromolecules which occurs in several microorganisms in the form of intracellular storage granules in response to nutritional and environmental conditions. Polyhydroxybutyrate (PHB) is a type of PHAs and its many material properties were similar to various synthetic polymers like polypropylene. However, the PHB differ from synthetic polymers in properties biodegradable, biocompatible and can be derived from renewable sources. These make them useful for extensive applications and future commercial large-scale production of PHB that can replace synthetic plastics, which currently obtained from petroleum bases[1].

Production cost is a major problem for PHB commercialization because it totally depends on the microorganisms, substrates (carbon source), the nutritional parameters, physical parameters and the downstream process such as recovery and purification. Currently, much efforts have been taken to reduce the PHB production cost through the strategies viz microbial strain development, optimizing fermentation process parameters and recovery processes[2,3]. The carbon source has the major influence over the (PHB) production cost, rummage around for inexpensive carbon sources has become one of the significant research in the PHB production[4]. Carbon rich wastes are attractive candidate since they have some of the desired characteristics namely low prices and high availability. A wide variety of substrates such as waste glycerol, whey, lignocellulosic materials and wastewater has been used with

different microorganisms to improve the yields of PHB production and also to avoid the environmental disposal problems[5,6].

The value-added products from feedstock of carbohydrate fractions present lignocellulosics is a promising strategy for sustainable bioprocesses. However, the necessity of obtaining monomeric fermentable sugars from carbohydrates represents an obstacle that still affects the economic viability of bioprocesses, especially due to the high recalcitrance of carbohydrate present biomass to pretreatment by chemicals or enzymes. A chemical pretreatment is an interesting approach of hemicellulose hydrolysis, resulting in a fermentable sugar enriched hydrolysate. Chemical pretreatment processes for hydrolysate preparation from hemicellulose by using dilute acid or alkaline solutions are commonly used and they further enhance enzymatic hydrolysis of the remaining cellulose. However, the chemical pretreatment requires neutralization and removal the chemical from hydrolysate to avoid negative effects on downstream steps[7,8]. Besides, during the pretreatment the phenolic and furan compound tends to become as a toxic compounds to microorganisms that are involving in bioprocesses[9]. Thus, challenges in the chemical pretreatment processes include preparing less toxic hydrolysates for microorganisms and less corrosive to process equipment. Therefore, optimization of chemical pretreatment of biomass are necessary [8,10,11].

Cost of biopolymer production is one of the most important factors restricting the industrial application of PHB. Thus, the main aim of this present study is to explore the feasibility of PHB biosynthesis which was evaluated with delignified giant reed grass hydrolysate as substrate and mutant bacterial strain as a biocatalyst under aerobic microenvironment. Next, the classical method of optimization was used for optimize the various physical parameters for enhancing PHB production from hydrolysate of giant reed grass. Further, the anticancer drug encapsulation efficiency and *in vitro* drug release was also studied to enhance the produced PHB application in pharmaceutical field. This is the first report to our knowledge claiming giant reed grass hydrolysate as a substrate for production of PHB.

MATERIALS AND METHODS

Materials and Chemicals

Giant reed grass (*Arundo donax* L.) sample was collected directly from a riverbank of Palaur river Kanchipuram, Tamil Nadu, India. The leaves of the sample plant were separated from the stems and then thoroughly washed with water to remove sand and other impurities. Then the sample was chopped into small pieces (size 1 mm) using lab scale blender and dried at 50°C for 12 h[12]. The dried sample was ground and stored in the fridge until use.

All chemicals used in the present study was highest purity or analytical grade (Hi-media Pvt. Ltd. Mumbai, and SRL India), obtained from recognized chemical supplier. The anticancer drug doxorubicin purchased from local medical shop.

Alkaline Pretreatment and delignification of giant reed grass (GRG) biomass

GRG sample was treated with different concentration of NaOH range from 1-3% at room temperature, microwave irradiation (2450MHz) (MI) and high temperature and pressure (HTP). The pretreated samples were washed with distilled water and then dried at 50–60°C for 24 h. Then the samples were subjected to delignification by sodium chlorite/acetic acid (SC/AA) method[13]. Finally, the samples were washed thoroughly with tap water to remove traces of chemicals used during the treatments and dried at 50–60°C before using for biosynthesis of PHB. Monosaccharide concentrations of sample each pretreated method was estimated. Based on the monosaccharide yield the best pretreatment condition was used for further study.

Bacterial strain and Maintenance

The feasibility of PHB biosynthesis was evaluated with GRG hydrolysate as substrate and mutant bacterial strain as a biocatalyst under aerobic microenvironment. In the present study, the mutant bacterium *Bacillus subtilis* RNM was used for PHB production. Before using, the bacterial strain was maintained in nutrient agar (Peptone 5 gL^{-1}, Beef extract 3 gL^{-1}, Sodium Chloride 5 gL^{-1} and Agar 15 gL^{-1}) slant and stored at 4°C for further uses. The cultures were revived after every month[14].

Biosynthesis PHB using GRG hydrolysate

The batch experiments were carried out in 250 mL shaking flasks using 100 mL of nitrogen limited (0.25 g/L NH_4Cl) mineral salt medium (MSM containing: Na_2HPO_4 (6 gL^{-1}), KH_2PO_4 (3 gL^{-1}), NaCl (0.5 g L^{-1}), $CaCl_2.2H_2O$ (1 M L^{-1}), $MnSO_4.7H_2O$ (1 M L^{-1}), $FeCl_2$ (0.01g L^{-1}), $CuSO_4.5H_2O$ (0.01g L^{-1}), $ZnSO_4.7H_2O$(0.1g L^{-1}), $MgSO_4.7H_2O$ (1 M L^{-1})) with maximum sugar contained GRG hydrolysate. Then flasks were inoculated with 1% of overnight culture of *B. subtilis* RNM and grown for 72 h in a rotary shaker at 30 °C and 200 rpm. Triplicate flasks experiments were carried out for each study. End of the experiment the samples were withdrawn and the biomass was separated by centrifugation at 14,000×g at for 15 min. The biomass was used to extract and estimated the PHB. The optimum concentration of GRG hydrolysate was used for further optimization of PHB biosynthesis with the following conditions. The effect of different pH (4.5 to 9), temperature (30 to 60°C) and agitation speed (100 to 250 rpm) on PHB biosynthesis by *B.subtilis* RNM was studied by classical method of optimization. All the experiments were carried out in triplicate to check the reproducibility.

Analytical methods

Estimation of total reducing sugars and HPLC analysis of monosaccharide

The sugar concentration of hydrolysates and culture supernatant was estimated by Dinitrosalicylic (DNS) acid method[15]. HPLC analysis of monosaccharide composition of hydrolysate was determined by Li et al[16]. Before HPLC analysis, 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatization of GRG hydrolysate was carried out by the method of Zhang et al[17]. Then the analysis was carried out with an HPLC using a Capcell Pak C_{18} MG column (3 mm i.d. x 250 mm, 5 µm, Shiseido, Japan) at 30°C. Elution was with sodium phosphate buffer (40 mM, pH 8.0)/acetonitrile (79:21, v/v) at 0.5 mL min^{-1}. Detection was at 245 nm.

Determination of cell dry weight (CDW)

The *B. subtilis* RNM cells were harvested by centrifugation at 14,000 rpm for 10 min. The pellet was collected and washed with distilled and frozen overnight at -20°C. Then the cells weight was determined and the CDW was expressed in gL^{-1}.

Extraction and estimation

PHB was extracted by the method of Koller et al[18]. *B. subtilis* RNM biomass was collected by centrifugation at 14,000 rpm for 10 min and the hydrophobic residual oil was removed

from biomass by treated with 5 ml of cold water and 2 ml of cold hexane. Then the biomass was dried in oven until getting a constant weight. About 3 g of dried biomass was used for Soxhlet extraction using chloroform as solvent. 5 mg of extracted PHB was redissolved in 5 ml of chloroform in water bath at 90°C for 25 min to remove the impurities and then filtered the sample using Whatman No.1 filter paper. Finally, chloroform was evaporated by taking the sample sterile glass petri plate and then stored at 4°C until further use [19]. The extracted PHB estimated by Law and Slepecky[20] method and used for further characterization.

Differential scanning calorimetry (DSC) analysis

The thermal transitions of PHB were measured using differential scanning calorimeter (TA Instruments Q-100, USA). The measurement was done over a temperature range from -20 to 180◦C at a heating rate of 10°C min^{-1} under nitrogen atmosphere (50 ml min^{-1}). The sample (2 mg) was in an aluminum pan and heated temperature range from -20 to 180°C at a scanning rate of 10°C min^{-1} under nitrogen atmosphere (50 ml min^{-1}). The thermal property of the sample was analyzed from the second heating round DSC curve. Glass transition (Tg) temperature was used as the midpoint of sample heat capacity change. Melting temperature (Tm) was estimated from DSC endothermal peaks. The maximum peak temperature was taken as T_m.

Thermogravimetric (TGA) analysis

TGA was performed on a Perkin-Elmer TGA 4000. The temperature was ramped at a heating rate of 10°C min^{-1} under nitrogen flow rate of 20 mL min^{-1} to a temperature well above the degradation temperature of the polymers (500°C)

Fourier transforms infrared spectroscopy (FTIR)

4 mg of PHB was mixed thoroughly with KBr (Spectroscopic grade) and then dried at 100°C for 4 h. IR spectra of PHB was recorded and analyzed using a single beam Perkin Elmer (Spectrum BX series, Sweden), with the scan in the range from 4000–500 cm^{-1}; number of scans, 16 and resolution, 4.0 cm^{-1}.

NMR analysis of PHB

The ^1H NMR spectra of sample (20 mg mL^{-1}) was recorded at room temperature in $CDCl_3$ using Bruker AV 400 NMR spectrometer (Bruker, Swiss) and determined the monomer composition of PHB using Kato et al., [21] method. The internal standard was Tetramethylsilane.

Preparation of Doxorubicin - PHB microparticles

PHB produced from GRG hyrdolysate by *B. subtilis* RNM was used to study the drug encapsulation efficiency and releasing efficiency. Anticancer drug doxorubicin - PHB microparticles were prepared by the oil-in-water emulsion-solvent evaporation method[22] with minor modification. A 500 mg of PHB obtained from GRG hydrolysate and the 400 mg of drug (200 mL) were dissolved in 5 mL of dichloromethane (DCM) and then emulsified in 200 mL of an aqueous phase containing 0.15 % (m/v) of poly (vinyl alcohol) [PVA] as stabilizer. Finally, the organic solvent (DCM) was evaporated by continuous stirring at 700 rpm, at room temperature. The doxorubicin loaded PHB microparticles were washed with distilled water followed by dried and stored under vacuum at room temperature. This preparation was repeated minimum three to four times to establish the reproducibility of this microparticles preparation.

Determination of drug encapsulation efficiency

The encapsulation efficiency of doxorubicin in PHB microparticles was estimated using UV-Visible spectrophotometer (Shimadzu-1700) at 266 nm by the method of Bazzo et al[22]. The microparticles drug content was then estimated. The loading efficiency was obtained using the following equation

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Drug found in microparticles}}{\text{Drug initially added to the formulation}} \times 100$$

***In vitro* release profile of Doxorubicin - PHB microparticles**

In vitro release studies of a doxorubicin loaded PHB nanomedicine formulation were carried out by dialysis bag diffusion technique with phosphate buffer solution (PBS) pH 7.4 at 37 ± 0.5 °C under constant stirring (100 rpm). A 10 mg of doxorubicin - PHB microparticles was enclosed in the dialysis bag (Dialysis membrane, molecular weight cut off of 12000-14000 Da (Himedia, Mumbai, India) and then incubated in 200 mL of PBS. At pre- selected time (0.5 to 72 h), 2 mL of sample was withdrawn and the drug released was measured by UV-Vis spectrophotometry, at 266 nm.

Statistical analysis

The results of all the experiments were presented as the mean ± standard deviation (SD) values of three independent replicates. The standard deviation did not exceed 5% of the average values. The one-way analysis of variance (ANOVA) also done for the obtained data using MINITAB 15 software at the significant level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Optimization of alkaline pretreatment for GRG hydrolysate preparation

Effect of different concentration of NaOH on pretreatment of GRG biomass at RT, MI and HTP was studied. After the pretreatment by each method, the hydrolysates were subjected to delignification treatment using the SC/AA. When increasing the NaOH concentration from 1% to 2% the amount of total reducing sugar (TRS) yield was increased. However, on further increase in the NaOH concentrations there was no changes in the yield of TRS. This result indicates that the NaOH concentration of 2% was the optimum level for pretreatment of GRG biomass, whereas the influences of conditions such as RT, MI and HTP on yield of TRS differ from each other. Because, in this study, the highest TRS of 416.82±0.04 mg g^{-1} and the lowest TRS concentration of 211.33±04 mg g^{-1} was obtained from GRG biomass after lignin removal by HTP and TR condition respectively (Table 1). At MI condition, the maximum TRS concentration of 338.66±0.03 mg g^{-1} was obtained after lignin removal from GRG biomass.

Table 1. Effect of different pre-treatment conditions on yield of total reducing sugars

NaOH Concentration (%)	Total reducing sugar (mg g^{-1} w/w) at		
	RT	MI	HTP
Control	180.2	281.2	384.21
1.0	200.2	320.12	408.21
1.5	210.32	334.25	410.32
2.0	211.32	338.66	416.82
2.5	211.33	338.62	416.86
3.0	211.45	338.63	416.89

Monosaccharide concentrations of lignin removed hydrolysate

The compositional analysis of different hydrolysates prepared from GRG biomass showed in the Table 2, it contains predominantly glucose and xylose but it also contained significant amount of arabinose and mannose. However, the yield of monosaccharides was high in the hydrolysate, which was obtained from GRG biomass by HTP pretreatment with 2% NaOH.

Table 2. Monosaccharide concentrations (%) of lignin removed hydrolysate obtained from GRG biomass after different pretreatment with 2% NaOH. Compositional analysis of hydrolysates was carried out by HPLC.

Treatment condition	Monosaccharide Composition (%)			
	Glucose	Xylose	Arabinose	Mannose
RT	71.24	24.31	7.33	1.95
MI	77.11	20.41	9.41	2.45
HTP	79.32	21.32	10.21	2.34

Selection of hydrolysate for PHB biosynthesis

The hydrolysates prepared from GRG biomass by all these three conditions (RT, MI and HTP) were checked its suitability for PHB biosynthesis using *B. subtilis* RNM. As shown in the Figure 1, the maximum PHB of 33.05±1.0 gL^{-1} and 34.25±0.43 gL^{-1} was obtained from hydrolysates prepared with 2% NaOH at MI and HTP conditions respectively. This yield ~1.8 fold higher than the PHB obtained from hydrolysate prepared with 2% NaOH at RT. However, the maximum PHB (34.25±0.43 gL^{-1}) production was observed in the GRG hydrolysate prepared with 2% NaOH at HTP. The bacterium *B. subtilis* RNM used in the present study was metabolically versatile because it utilized all the sugars of this hydrolysate effectively for growth and PHB production. Hence, this condition of pretreatment was found to be an optimum pretreatment condition for hydrolysate preparation from GRG biomass. Further, this pretreatment condition was used throughout this study for hydrolysate preparation. This study demonstrates that the combined physicochemical pretreatment is the best method for extracting TRS for various applications. A critical and important feature of PHB production process is the assimilation and utilization of these sugars by microorganisms. Generally, the bacterial strains have the ability to produce biodegradable polymers from different organic substrates[23,24]. Based on the experiment outcomes, the GRG hydrolysate prepared by HTP pretreatment with 2% NaOH was considered as best feedstock for maximum PHB biosynthesis using *B. subtilis* RNM.

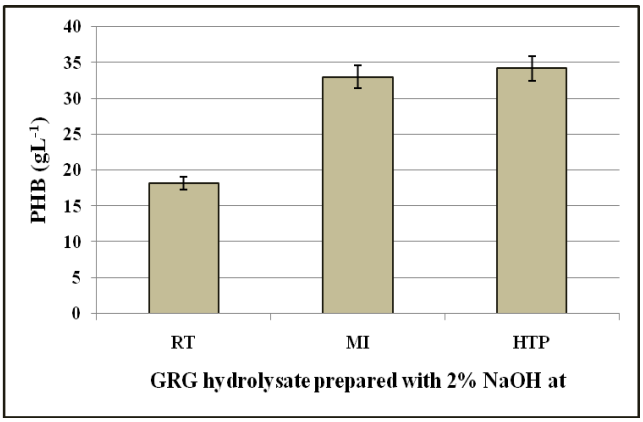

Figure 1. Effect of hydrolysate prepared by different pre-treatment conditions on the biosynthesis of PHB using *B. subtilis* RNM

Effect of pH on biosynthesis of PHB

The effect of medium initial pH on the biosynthesis of PHB from GRG hydrolysate using *B. subtilis* RNM was evaluated with different pH range from 4.5 to 9 with an increment of 0.5 pH. The production medium pH conditions were maintained using 1N NaOH/HCl. As shown in the Figure 2, the maximum PHB production was observed between the pH 6.5 to 7.5, however the highest PHB of 43.22±0.42 gL^{-1} was obtained at pH 7.0. The pH below 6.5 and pH above 7.5 were severely affected the growth of *B. subtilis* RNM, since the PHB yield was decreased. In the current study, pH 7 was found to be an optimum pH, which is similar to the finding of Kulpreecha et al[25] who obtained the maximum PHB production by new isolate *B. megaterium* at pH 7.0.

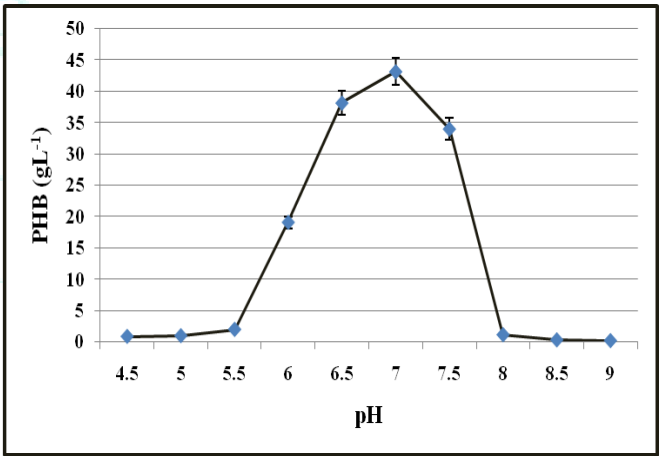

Figure 2. Effect of pH on biosynthesis of PHB from GRG hydrolysate

Effect of incubation temperature on biosynthesis of PHB

Effect of the incubation temperature on biosynthesis of PHB from GRG hydrolysate using *B. subtilis* RNM was studied. The cultures were maintained at different temperature range from 30°C to 60°C with an increment of 5°C. As shown in the Figure 3, the maximum 39.35±0.33 gL^{-1} of PHB production was obtained from hydrolysate using *B. subtilis* RNM at 35°C. This temperature was found to be the optimum temperature for enhancing the PHB production from GRG hydrolysate by *B. subtilis* RNM. Further, increase in the incubation temperature is unfavourable for the growth of *B. subtilis* RNM, hence decreases the PHB production. The correlation value of 0.963 (P<0.001) was found for biomass and PHB production. This suggests that the biomass and PHB are

interdependent. Similar correlation values were found for the biomass and temperature, therefore these two is in correlation. The result suggesting that the temperature above 35°C do not support the growth of *B. subtilis* RNM.

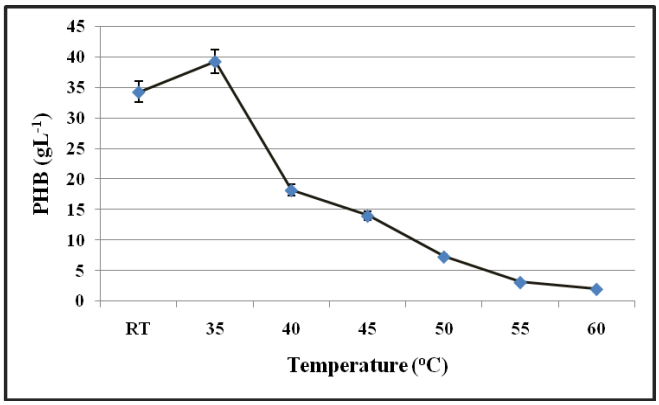

Figure 3. Effect of temperature on biosynthesis of PHB from GRG hydrolysate

Effect of agitation speed on biosynthesis of PHB

Effect of agitation speed on the biosynthesis of PHB from hydrolysate using was evaluated by varying the culture agitation speed from 100 to 300 rpm with an increment of 50 rpm at optimum pH 7.0 and temperature 35°C. When increasing the agitation speed from 100 to 150 rpm there is increase in the PHB production (Figure 4), but further increase in the speed there was no increment in the PHB production because the excessive shear force developed at agitation speed >150rpm.

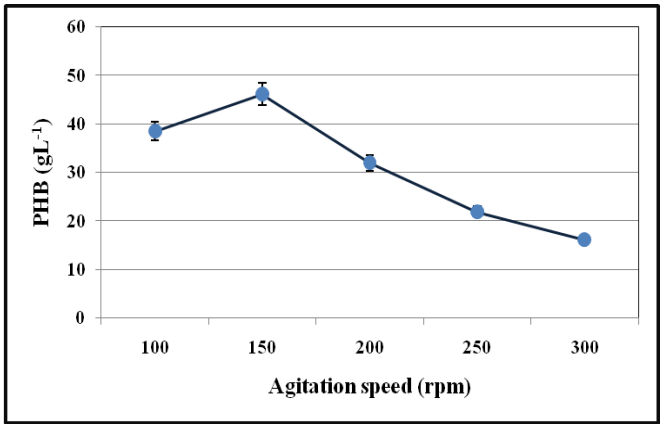

Figure 4. Effect of agitation speed on biosynthesis of PHB from GRG hydrolysate

Characterization of PHB obtained from GRG hydrolysate

^{1}H NMR characterization

The ^{1}H NMR spectrum of PHB was obtained with $CDCl_3$ solution. In Figure 5, methyl protons (-CH3) appear to have a single resonance at 1.5 ppm, methylene protons (-CH2) appear to have a multiplet resonance at 2.45 ppm, methine proton (-CH) of bacterial polyhydroxybutyrate also has a multiplet resonance at 5.32 ppm. The ^{1}H NMR spectra implied that the PHB contains two monomeric units HB and HV.

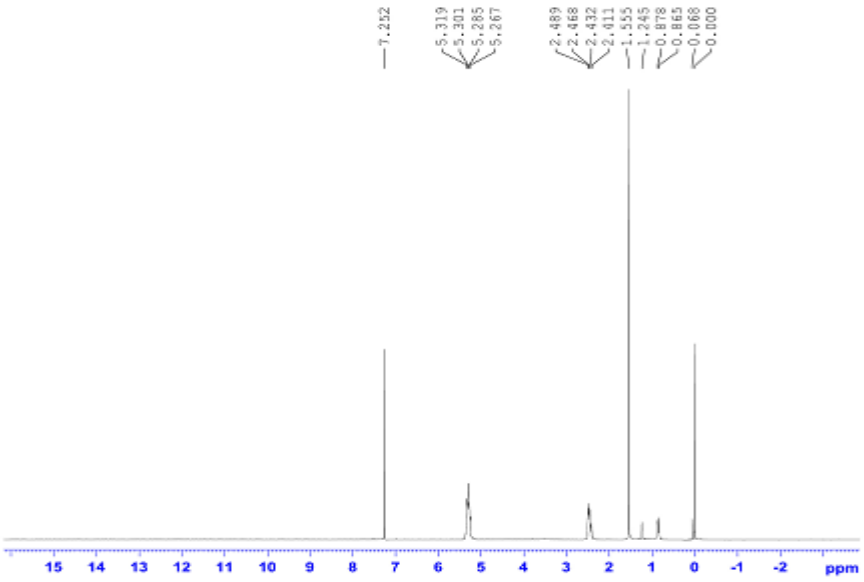

Figure 5. ^{1}H NMR spectrum of PHB biosynthesized from GRG hydrolysate

FTIR spectroscopy analysis

The PHB extracted from GRG hydrolysate grown *B. subtilis* RNM was resembled with commercial PHB because the PHB marker absorption peak 1731.2 cm^{-1} was obtained which is for characteristic of carbonyl ester (RC=O). Similarly the other absorption peaks in the Figure 6, 3416.20 cm^{-1}, 2921.80 cm^{-1}, 2634.50 cm^{-1}, 1414.10 cm^{-1} and 1192.80 cm^{-1} were indicative of the presence of hydroxyl group[26] (Ma *et al.*, 2009), asymmetric methyl group, CH_2 group, CH_3 group[27] and C-O group in the polymer chain of extracted PHB which is similar to the FTIR absorption peak of commercial PHB.

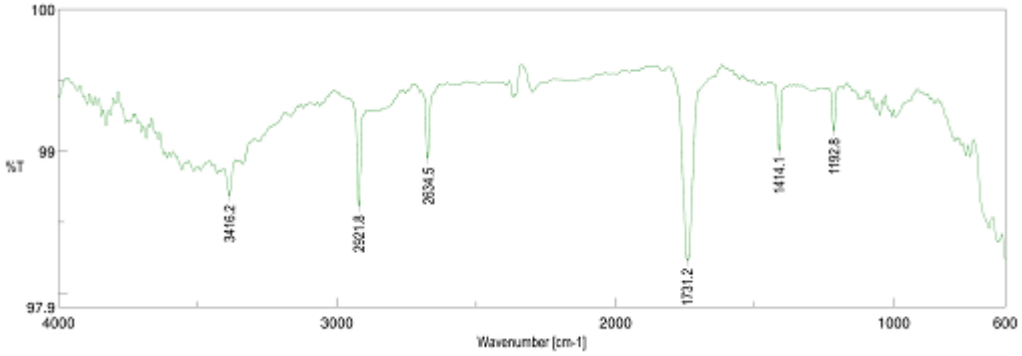

Figure 6. FTIR spectrum of PHB biosynthesized from GRG hydrolysate

Thermal analyses

Thermal properties of polymer such as Tg and Tm are crucial for polymer processing. The Tm of the present study PHB was found to be 171.02 °C (Figure 7). Previous reports on Tm of different microbial PHB extracted under different extraction condition were range from 171–180 °C [28,29,30,31], which is in conformity with this finding. Figure 8, represented TGA profile of PHB produced from GRG hydrolysate. A rapid thermal degradation was observed between 249 °C and 280 °C with a peak at 275 °C. TGA profile of PHB obtained from GRG hydrolysate by *B. subtilis* RNM showed that the thermal stability with complete degradation occurring at 275 °C, which is above the melting temperature of 179.5 °C of the pure PHB[32]. The difference between decomposition and Tm of produced PHB was high enough to permit for further processing. The PHB obtained from *B. subtilis* RNM could be used for various biomedical applications.

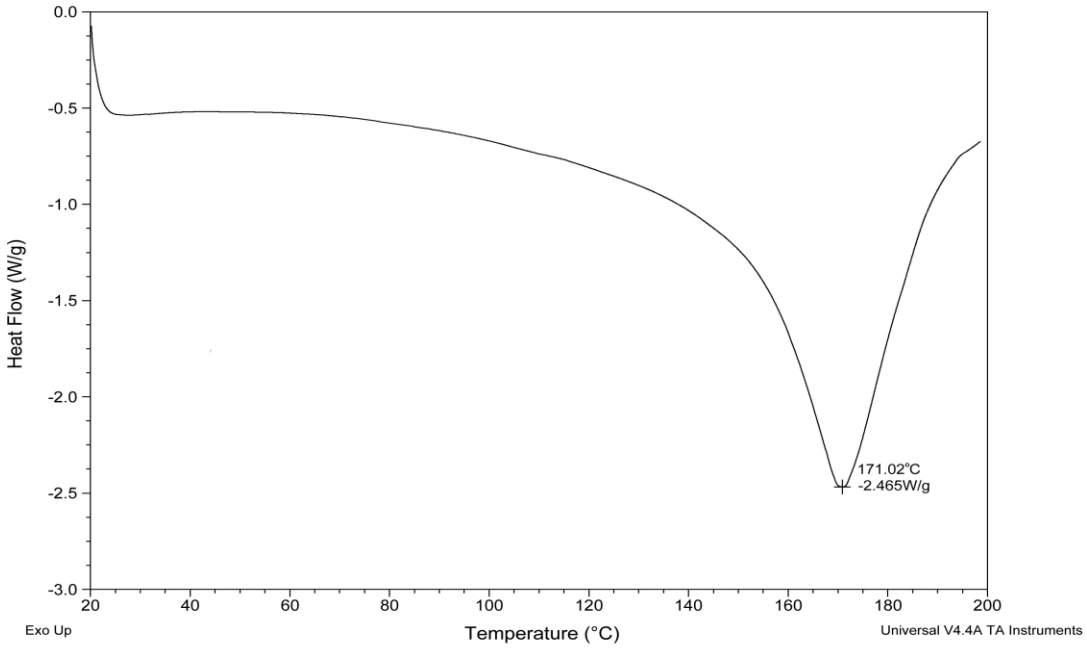

Figure 7. DSC of PHB biosynthesized from GRG hydrolysate

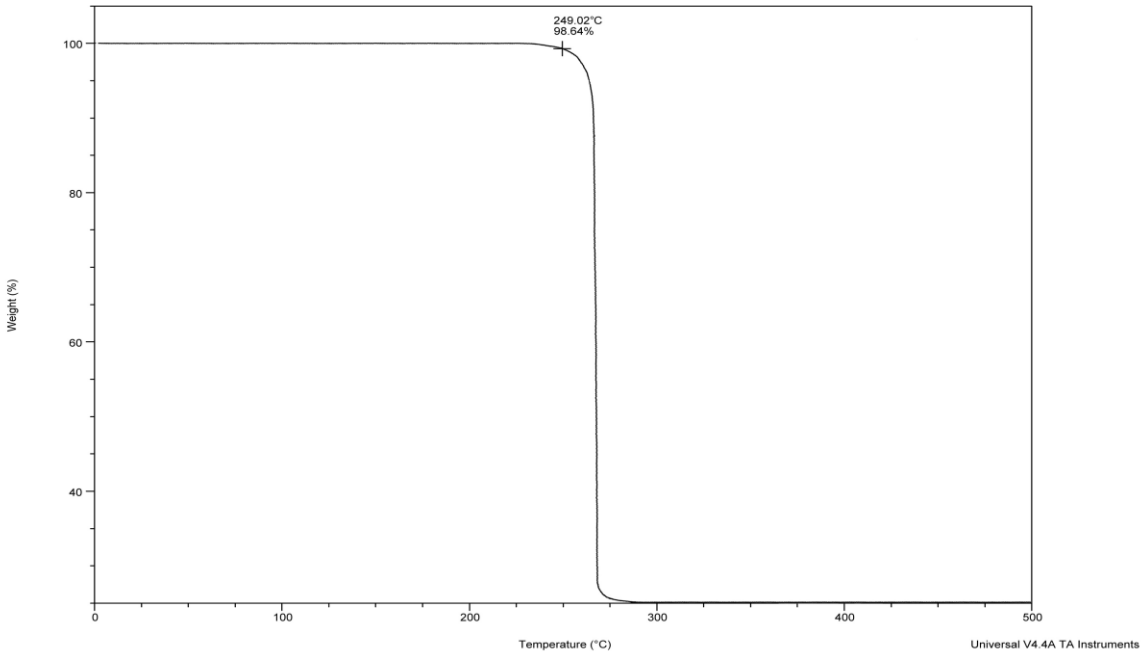

Figure 8 TGA of PHB biosynthesized from GRG hydrolysate

Preparation and i*n vitro* release profile of Doxorubicin - PHB microparticles

Doxorubicin is well known anticancer chemotherapy drug and used for different kinds of cancers like Breast cancer, gastric cancer, liver cancer, ovarian cancer, uterine sarcoma etc. One of the main side effect of this drug is causes heart failure. Doxorubicin is directly administered into the coronary vessels to minimize systemic side effects such as alopecia, weight loss, colitis, and bone marrow suppression[33]. In this study, doxorubicin - PHB microparticles were successfully prepared and found 93.21±0.15% as encapsulation efficiency of drug doxorubicin in PHB microparticles. Generally, drug entrapment efficiency in polymers or polymer matrix completely depends on the nature of polymers, solid-state drug solubility in the polymers and end functional groups (ester or carboxyl)[34,35,36,37].

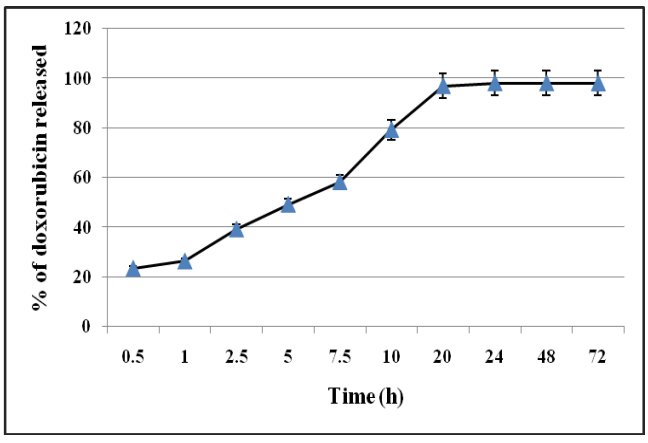

Figure 8. *In vitro* release profile of doxorubicin from PHB microparticles

Further, *in vitro* release studies of a doxorubicin loaded PHB nanomedicine formulation were carried out by dialysis bag diffusion technique. As shown in the Figure 8, after 24 h, 98% of the encapsulated doxorubicin was released from the microparticles. The PHB used for this study, was biosynthesized from GRG hydrolysate. These results indicate that the PHB biosynthesized from GRG hydrolysate using *B. subtilis* RNM, could be a best biodegradable polymer for drug encapsulation and delivery.

CONCLUSION

The HTP pretreatment with 2% NaOH was very effective to convert the GRG biomass into fermentable sugar for PHB production because at this condition the maximum glucose of 79.32% was obtained after lignin removed by SC/AA treatment. The PHB yield increased from 34.25±0.43 gL^{-1} to 46.21±0.2 gL^{-1} when *B. subtilis* RNM grown under optimized condition using GRG hydrolysate as low cost PHB biosynthesis medium. This study suggests using the GRG in substitution for commercial medium, reducing PHB production costs and increasing PHB yield. Moreover, the ^{1}H NMR and FTIR characterization of the PHB biosynthesized from GRG hydrolysate using *B. subtilis* RNM, results revealed that the PHB was similar to the commercial PHB. Besides the thermal analysis Tg and T_m also results were also similar to the commercial PHB. The produced PHB have 93.21±0.15% of anticancer drug doxorubicin encapsulation efficiency in PHB microparticles and *in vitro* condition, 98% of the encapsulated doxorubicin releasing efficiency. Hence, the PHB biosynthesized from GRG hydrolysate using *B. subtilis* RNM, could be a best biodegradable polymer for anticancer drug encapsulation and delivery.

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