



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student : Chethana M

Roll Number : 166107103

Programme of Study : Ph.D.

Thesis Title: Studies on Development of Bio-based Biodegradable Polymers and Their Efficacy as Biomaterials

Name of Thesis Supervisor(s) : Prof. Vimal Katiyar & Dr. Amit Kumar

Thesis Submitted to the Department/ Center : Yes

Date of completion of Thesis Viva-Voce Exam : 16/05/2023

Key words for description of Thesis Work : Polyhydroxybutyrate, Poly(lactic acid), Nanocellulose, Prodigiosin, Biomaterials

SHORT ABSTRACT

Exhaustion of non-renewable resources and increasing concern towards the non-biodegradability of conventional polymers demands research for alternative, bio-based and biodegradable polymers having comparable properties. Recently, research in the field of sustainable polymers is highly focused on the use of renewable wastes generated from agriculture, food, and the meat industry for the development of biodegradable biomaterials. The biomaterials prepared from these renewable wastes find their applications in the field of medicine and help to restore biological functions. Polyhydroxybutyrate (PHB) is a biodegradable biomaterial of bacterial origin produced intracellularly as an energy reserve in a stress-induced condition which has a potential application in drug delivery. Poly(lactic acid) (PLA) is another class of bio-based polymer produced by the polymerization of lactide predominantly used in biomedical applications and packaging. Prodigiosin is a bacterial pigment used as a remedy for cancer treatment, and nanocellulose is a bionanomaterial suitable for high-performance applications. In this perspective, the current study extensively deals with the utilization of waste sorghum stalks consisting of fermentable sugars, cellulose, and hemicellulose for the production of biomaterials such as PHB and nanocellulose. Prodigiosin is produced from *Serratia nematodiphila* isolated from river water, and metal-free PLA is synthesized using prodigiosin as a metal-free catalyst. Over the assessment, the juice from agricultural waste sorghum stalks consisting of ~60 g/L of fermentable sugar is used as an inexpensive carbon source for the production of PHB. The minimal media supplemented with sorghum juice is used for the optimization and production of PHB. The optimized conditions yielded the maximum productivity of ~8.2 g/L of PHB within 24 h of cultivation. The fed-batch operation with dO_2 controlled strategy maximized the productivity four-folds in comparison to the batch operation. It was able to obtain PHB with a molecular weight of ~400 KDa and recovery of ~94% using solvent extraction. The characterization of the produced

PHB exhibited improved crystallization temperature (T_c) from 101 to 105 °C, glass transition temperature (T_g) from -8 to -10 °C, melting temperature (T_m) from 162 to 175 °C, maximum degradation temperature (T_{max}) of 318 °C, and wettability from 79 to 93 °C which are comparable to commercial PHB. Based on this assessment, the produced PHB is utilized for the preparation of microspheres using the solvent evaporation method. The morphology and size of the microsphere could be tuned from 7-300 μm by varying the stirring speed. To check its efficacy as an embolizing agent for minimally invasive treatments, a low-cost 3D printed PLA-based hepatic phantom costing 221 INR was developed and evaluated for its suitability as an *in vitro* model for simulating the right hepatic artery. Further, the unique setup has been developed to mimic the systolic and diastolic blood pressures with a flow rate ranging from 60 to 120 mL/min. The inline microscopic setup helps in the tracking of the flow of the microsphere inside the channels and at the junctions. The study also confirmed the embolization effect for the 0.9 mm channel based on the microsphere size.

Another objective of this thesis is focused on the production of organic small-molecule prodigiosin (PG). A detailed study on the production of prodigiosin revealed a maximum yield of $\sim 0.37 \pm 0.04$ g/g of cell biomass at optimum conditions with a molecular mass of (m/z) 323.6 Da, and a characteristic absorption wavelength λ_{max} of 535 nm. Further, the antibacterial test conducted with prodigiosin against *Staphylococcus aureus* confirmed its antibacterial ability. The produced prodigiosin is also utilized as an organocatalyst for ring-opening polymerization (ROP) of L-lactide to produce a metal-free PLA of molecular weight of ~ 5000 Da. This range of molecular weight is more suitable for drug delivery applications. However, commercial PLA is susceptible for bacterial adhesion which in turn leads to infections. This remains an obstacle in implementing PLA-based implants. To tackle this issue, prodigiosin incorporated PLA has been developed, which possesses an improved hydrophobicity with a contact angle of $111 \pm 1.5^\circ$. The degradation temperature of the prodigiosin is 215 °C, which is more than the melting temperature of PLA supports the processability and sterilization of the PLA-based implants without any toxic gases. Further, the prodigiosin improved the transparency of the PLA and acted as a nucleation site. The *in vitro* study on biofilm formation shows excellent inhibition activity against implant-associated pathogens such as *Klebsiella aerogenes* and *Staphylococcus aureus*. On the other hand, leftover sorghum bagasse after extraction of juice is utilized for the fabrication of nanocellulose using alkaline hydroxide peroxide treatment. The current method utilized the high-pressure treatment at 15 lb and it could remove $\sim 97\%$ of the lignin. Further, the obtained cellulose was hydrolyzed with sulfuric acid at different time intervals to produce the nanocellulose of 33 ± 4 nm to 560 ± 126 nm in size. This process also yielded ~ 50 g/L of xylose and 7 g/L of glucose, indicating the breakdown of hemicellulose as well as cellulose, respectively.

The cytotoxicity test performed on baby hamster kidney fibroblast cells (BHK-21) confirmed the non-toxicity of all the developed biomaterials. Overall the developed materials such as PHB, PLA, and prodigiosin successfully demonstrated their ability as potential drug carriers, organic catalysts, biocompatible materials, antibiofilm-forming agents, and embolizing agents for hepatic cancers.