ABSTRACT

The ever increasing population has led to accumulation of non degradable waste materials in the environment affecting the very survival of the living world. Petro-derived plastics are widely used to make a variety of industrial and consumer products. But their intrinsic durability and resistivity towards degradation renders them pollutants in the environment. There is no effective method for the degradation of these recalcitrant materials. Considering the huge economic and ecological costs involved in the ever depleting environment from such petro-derived plastics, the idea of substituting non-degradable synthetic plastics with ecofriendly biodegradable plastics has drawn considerable attention world over. Among the various biodegradable plastics available, polyhydroxyalkanoate (PHA) is a promising natural polyester produced by bacteria as their intracellular storage energy material while suffering from a particular stress. PHA is not only biodegradable and biocompatible, but also known to possess same physical properties as that of conventional plastics thereby making it suitable for the various applications.

It is known that the petroleum hydrocarbon contaminated soils harbor a large number of microbes constituting an environment of excess carbon with limited nitrogen, and as such these sites could be the potential source for PHA producing bacteria. Assam is rich in petroleum and the first oil well was discovered in Digboi more than a hundred years ago. The state has over 1.3 billion tones of proven crude oil and 156 billion cu mt of natural gas reserves. The premier Indian Oil companies like ONGC, OIL and IOCL are involved in exploration, development and production of crude oil and natural gas, transportation of crude oil and production of LPG. In view of the above background information, the present investigation was taken up with the following objectives:

1. Screening of biopolymer producing bacteria
2. Isolation and purification of PHA
3. Physical and chemical characterization of PHA
4. Molecular genetic assessment of PHA producing bacterial isolates

Bacterial strains were isolated from the soil samples collected from crude oil contaminated sites of Assam and Assam Arakan Basin, ONGC; waste disposal site of Numaligarh Refinery Limited, Assam and non-petroleum industrial site of Jagiroad Paper Mill, Nagaon, Assam for the isolation of PHA producing bacteria. A
total of 13 pure bacterial strains were isolated and screened for PHA production. The standard staining procedures enabled selection of 3 isolates such as BPC1, BPC2 and BP2 for assessing their potential PHA production.

Morphological characterization (colony size, pigmentation, forms, margin and elevation) displayed wide variations. All biochemical tests were carried out to characterize the bacterial strains. The optimum growth conditions of the bacterial isolates were assessed by subjecting them to different growth phase, pH and temperature regimes. The optimal conditions for PHA production were- pH 7.0, 37 °C temperature and 72 h culture period.

For molecular characterization of the biopolymer producing bacterial isolates BPC1 and BPC2, 16s rDNA analysis was performed. Genomic DNA isolated from the bacterial isolates was used for the amplification of 16S rDNA by PCR and then sequenced. Subsequently, data generated were deposited in the GenBank of NCBI, compared with published sequences and phylogenetic tree was constructed using ClustalW by the distance matrix analysis and the neighbour-joining method. Phylogenetic analysis indicated a comparative search for the sequence of BPC1 and BPC2 revealed 99% homology to other known Pseudomonas aeruginosa 16S rDNA gene sequences and accordingly they were named as *P. aeruginosa* BPC1 (JQ796859) and *P. aeruginosa* BPC2 (JQ866912), respectively. The bacterial strain BP2 was previously identified by our laboratory and named as *Bacillus circulans* MTCC8167.

The bacterial strains were subjected to growth kinetic study in the different culture media supplemented with the carbon substrates like glucose, glycerol, *colocassia* starch, propionic acid, soy oil, waste mobile, sugarcane molasses and glycerol byproduct of kitchen chimney dump lard (KCDL). The bacterial strain *P. aeruginosa* JQ866912 possessed the higher accumulation of PHA as compared to *B. circulans* MTCC8167 followed by *P. aeruginosa* JQ796859. Comparatively, when the glycerol byproduct (waste of KCDL) was used as the carbon source, *P. aeruginosa* JQ866912 showed the highest PHA accumulation in comparison to the other carbon sources, followed by *P. aeruginosa* JQ796859 and *B. circulans* MTCC8167. Similar results were obtained in the case of biomass yield by the bacterial strains with the use of the above stated carbon sources.
The biopolymer was isolated following the soxhlet extraction method with chloroform as the solvent, and purified by washing repetitively with ice cold methanol. Subsequently, the chemical and structural characterization was done using UV-Vis spectroscopy, FTIR, GCMS and \(^1\)H and \(^{13}\)C NMR methods. The results confirmed the isolated PHAs from *P. aeruginosa* JQ796859, *B. circulans* MTCC8167 and *P. aeruginosa* JQ866912 to be poly (3-hydroxyvalerate) co- (5-hydroxydecenoate) (P-3HV-5HDE), poly-3-hydroxybutyrate-co-3-hydroxyvalerate (P-3HB-3HV) and poly-3-hydroxyvalerate-co-5-hydroxydecenoate-co-3-hydroxyoctadecenoate (P-3HV-5HDE-3HODE) respectively. The molecular weight of the biopolymers was assessed by GPC and the value was found to be in the order *B. circulans* MTCC8167 > *P. aeruginosa* JQ866912 > *P. aeruginosa* JQ796859.

XRD analysis was carried out to study the crystalline behavior of the polymers. The degradation profile and melting point of the extracted biopolymers were tested by using TGA and DSC analysis, respectively. All three biopolymers possessed thermal as well as melting stability. The photoluminescence property of the biopolymers was studied by photoluminescence spectrophotometer (PL) and all of them showed luminescence property. SEM analysis was done to examine the surface morphology of the extracted biopolymers.

*In vitro* degradation of the biopolymers from *P. aeruginosa* JQ796859 and *P. aeruginosa* JQ866912 was done by treating with different soil bacteria like *Alcaligens faecalis* (MTCC8164), *B. circulans* (MTCC8167), *P. aeruginosa* (MTCC7815) and *Mycobacterium* spp (G-351); and the biopolymer from *B. circulans* MTCC8167, by *B. subtilis* strains BP4, BP5, BP7 and fungal strains *Candida albicans* (MTCC 3017) and *Fusarium oxysporum* (NCIM 1281). FTIR and SEM analysis was done to assess the pattern of degradation of the biopolymers. All these analyses supported biodegradability of the biopolymers when exposed to microbial action.

Silver nanoparticle (SNP) has been extensively used in biomedical studies, cosmetics, antibacterial-water filter and also as drug carrier. The natural hydrophobic biopolymers such as PHA could be an effective alternative and renewable source for the aforementioned applications. SNP synthesized from AgNO\(_3\) was impregnated by dispersing in PHA colloid (0.085%) using NaBH\(_4\). *In situ* formation of the SNP-PHA colloid was confirmed by FTIR analysis. The
stability was tested by wave length scanning using a UV-Vis spectrophotometer and finally with a transmission electron microscope (TEM). The composite of SNP-PHA was found to be stable for 30 days as compared to the lower stability of SNP solution alone. In the present investigation, the PHA polymer of *B. circulans* MTCC8167 was used for stabilizing the size of SNP particles which in turn would provide advantage for their application in synthesis, transportation of SNP colloids and their use in different biomedical formulations.

The change in photoluminescence properties in doping of the copper, zinc and nickel oxide nanoparticles with PHA polymer of *P. aeruginosa* JQ866912 was studied using XRD, UV-Vis spectroscopy and PL. XRD analysis and the same showed the successful incorporation of nanoparticles in the polymer matrix. The PL spectra of the materials showed a dramatic change in the emission nature to that of the virgin polymer.

Malaria is a vector-borne infectious disease and continues to be a major health problem in the tropics and subtropics. The antimalarial drug artemisinin has successfully been used in the treatment of growing resistance of *Plasmodium falciparum* for more than two decades. But there was a case of significant liver inflammation due to prolonged use of relatively high-dose of artemisinin. In view of the importance of artemisinin in treatment of malaria, several methods have been developed for quantitative analysis of its metabolites. But, none of the existing methods could determine the trace amount of artimisinin in the biological fluids like urine as well as in pharmaceutical formulations. As such there is a need for development of an efficient technique to detect the lowest possible traceable amount of artimisinin in biological fluids and pharmaceutical formulations. Accordingly, a highly sensitive method was developed using a selective biosensor comprised of PHA-gold nanoparticles (AuNPs) composite synthesized on indium–tin oxide (ITO) glass plate for direct determination of artimisinin in pharmaceutical formulations and biological fluids. The sensor was fabricated by adsorbing horseradish peroxidase (HRP) enzyme on the electrode surface for which cyclic voltametry (CV) was used to monitor the electro-catalytic reduction of artemisinin under diffusion controlled conditions. Electrochemical interfacial properties and immobilization of enzyme onto PHA-AuNPs film were evaluated and confirmed by CV, Electrochemical impedance spectroscopy (EIS) and SEM techniques. The tested biosensor exhibited
low LOD values, high levels of repeatability and reproducibility in pharmaceutical formulations and also in the spiked human serum. The PHA/AuNPs/HRP/ITO biosensor based nanocomposite probe has potential as a good analytical alternative in comparison to other existing methods presently available for such tests.

To determine the presence of PHA biosynthesis genes in *P. aeruginosa* strains JQ796859 and JQ866912, PCR based molecular technique was used for their elucidation. The amplification was carried out using two primer-pairs, I-179L and I-179R. The PCR product of 540 bp representing the partial coding sequences of the genes phaC1 and C2 was obtained. The same has evidenced for the presence of PHA biosynthesis genes in the concerned two bacterial strains.

References