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Title: Screening, Characterization And Optimization Of Polyhydroxybutyrate (PHB) From Cyanobacteria.

ABSTRACT

During the last century, petrochemical-based plastics appeared as one of our most practical daily use materials. This is due to their versatile properties that include durability and resistance to degradation. But these very desirable properties of plastics pose problems which can be cumulatively called as plastic pollution or white pollution. Thermoplastic processability, similar mechanical properties, complete biodegradability and biocompatibility of polyhydroxybutyrate (PHB), the most common representative of polyhydroxyalkanoates (PHAs) make it an attractive alternative to the common plastics. At present, PHB accumulation has been observed in a range of wild types and recombinant bacteria under fermentative processes but high production cost and complex recovery processes limits bacterial PHB production. Nevertheless, the exploitation of photosynthetic cyanobacteria for PHB has come up as an excellent way for biodegradable plastic production. 23 cyanobacterial strains (15 heterocystous and 8 non-heterocystous) were screened for PHB production. The highest PHB (6.44 % w/w of dry cells) was detected in *Nostoc muscorum* NCCU-442 and the lowest in *Spirulina platensis* NCCU-S5 (0.51 %% w/w of dry cells), whereas no PHB was found in *Cylindrospermum sp* NCCU-272, *Oscillatoria sp* NCCU-369 and *Plectonema sp* NCCU-204. *N. muscorum* NCCU-442 emerged as the best PHB-yielding strain and hence was selected for further experiments. Pretreatment of biomass with methanol: acetone: water: dimethylformamide [40: 40: 18: 2 (MAD-I)] with 2 h magnetic bar stirring followed by 30 h continuous chloroform soxhlet extraction acted as optimal extraction conditions. The optimum physico-chemical conditions were: pH 7.5, 30^{o}C, 10:14 light and dark periods with

0.4% glucose (as additional C source), P-deficiency and 1.0 gl^{-1} NaCl. Under these optimised conditions, *N. muscorum* NCCU-442 accumulated 26.37% PHB, as compared with 4.42% after 7 days. UV, HPLC, FTIR, NMR, GC MS, TGA and DSC analyses confirmed the extracted polymer as PHB after comparing with standard PHB (Sigma-Aldrich). The polymer showed good tensile strength and Young's modulus with a low extension to break ratio comparable to petrochemical plastic. The quantitative values for the three mechanical properties were calculated to be 31.1 MPa, 8.6 % and 1.5 GPa, respectively. Hydrophilicity of the extracted polymer film was determined by finding out the parameters like surface tension and contact angle and was determined to be less hydrophilic than standard PHB. PHB polymer film of *Nostoc muscorum* NCCU-442 showed efficient degradation (24.58 % weight loss ratio) within 60 days by mixed microbial culture unlike conventional plastic (1.68 %). In order to increase flexibility, thermoprocessability and biodegradability of the extracted polymer, it was blended with polyethylene glycol (PEG) to form PHB-PEG blends. The blends showed good miscibility between PHB and PEG. Elongation to break ratio (measured for determining flexibility) increased from 8.6% to 21.2% on PEG addition, thus increasing the extracted polymer's flexibility. The initial degradation temperature (T_{onset}) and melting temperature (T_m) of the blends decreased from 256 ^{o}C to 246 ^{o}C and 171 ^{o}C to 152 ^{o}C respectively with the increasing PEG content. But the difference of T_{onset} and T_m (denoted by ΔT, which is an important parameter for thermoprocessability) increased in all blends demonstrating their better thermoprocessability. Addition of PEG also increased their biodegradability (from 24.58% to 100% weight loss) after 60 days in the presence of soil microflora.Our findings suggested that cyanobacterial (*Nostoc muscorum* NCCU-442) PHB can be utilised as a good source of PHB based plastics that can replace or reduce petroleum based plastics in the environment.