

Polyhydroxyalkanoates (PHAs) are natural, renewable and biocompatible biopolymers which can be made into plastic materials with properties similar to petrochemical plastics.

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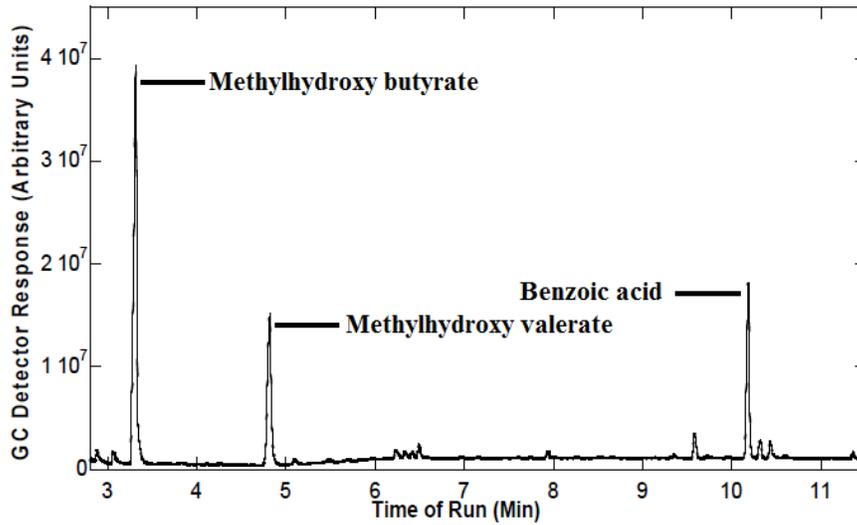
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Halophilic microorganisms are an important source of biopolyesters and hold promise for providing an economically competitive industrial scale production process. Vinasse, a recalcitrant waste of the ethanol industry was employed for the production of polyhydroxyalkanoate (PHA) by the extremely halophilic archaeon, *Haloarcula marismortui* in shake flasks. The PHA was recovered by osmotic lysis of the cells and subsequent purification by sodium hypochlorite and organic solvents. The PHA was found to have characteristics very similar to that of the standard polyhydroxybutyrate (PHB) from Sigma. Maximum specific growth rate, specific production rate, and volumetric productivity attained using 10% raw vinasse were comparable to that obtained using a previously reported nutrient deficient medium (NDM), while the values with 100% pre-treated vinasse were higher than that determined using NDM medium. This is the first report of polyhydroxybutyrate production by a halophilic microorganism utilizing vinasse.

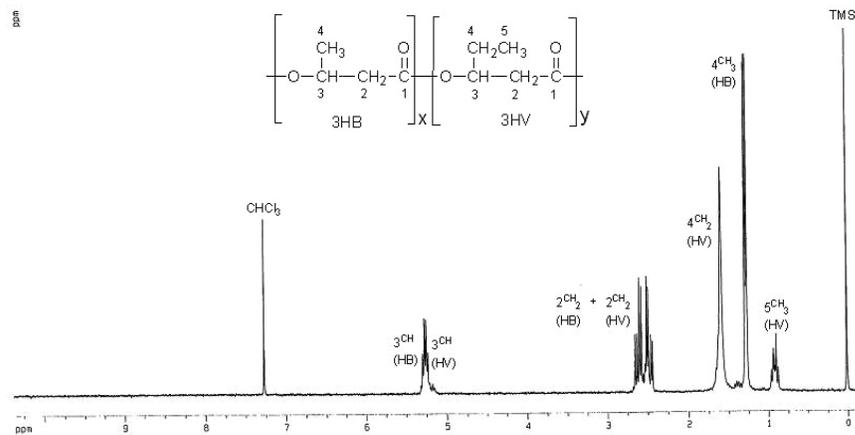
Vinasse was utilized for the production of polyhydroxyalkanoate (PHA) by the extremely halophilic archaeon, *Haloferax mediterranei*. Maximum PHA concentration of 19.7 g/l, product yield coefficient (based on total carbohydrates) of 0.87 and 0.21 g/l h volumetric productivity were achieved. Concomitant lowering of BOD₅ of pre-treated vinasse by at least 78% and COD by at least 80% was attained at the end of this process. The PHA was recovered by osmotic lysis of the cells and purification by sodium hypochlorite and organic solvents. The PHA was identified as poly-3-(hydroxybutyrate-co-hydroxyvalerate). High salt concentration in the medium allowed this process without sterile conditions and thus reduction in costs of sterilization can be envisaged. This process can easily be integrated into a distillery that has fermentation equipment and trained personnel.

In the second year, standardization of the PCR screening method for the detection of positive PHA accumulating halobacteria has provided us with a rapid and definite method of identifying PHA producers. Although a Chinese group cloned the PCR fragments after amplification of the *phaC* gene; the details of the PCR conditions were not described. We developed the method which will be useful to our future work as well as to other researchers.

The technology developed so far can have immediate applications. IFB Agro Industries (Kolkata) is interested to undertake pilot-plant studies. Use of bags made of the biodegradable plastic (PHBV) will not only be the first in India but also bring relief from the pollution caused by plastic bags.



Gas chromatogram of PHA obtained from *Haloferax mediterranei* utilizing vinasse



^1H NMR spectrum of PHA obtained from *Haloferax mediterranei* cultivated in vinasse