Discussion
This study has lead to the utilization of waste materials (that we have produced in tons but difficult to dispose in an eco-friendly manner) as a substrate for production of biodegradable plastic. The dual purpose of treating the waste and production of bioplastics has been achieved. Thus, making use of an inexpensive material and a novel process to produce biodegradable, biocompatible and user-friendly product of daily use and simultaneously keeping pollution levels under check and creating a healthy atmosphere for perpetuation of mankind. In order to exploit the PHB producing abilities of different microbes, it is desirable to produce it from cheap raw materials such as biological wastes. Since the first stage in bioconversion of biowastes into value added products involves hydrolysis of complex organic matter such as carbohydrates, proteins and fats, it is economical to use those microbes which also solublize the various constituents of the biowastes.

The first aim of this study was to isolate microbial isolates from fermentating biowastes (Potato peels incubated with cow dung slurry). Around 500 isolates were picked from nutrient agar plates at pH 2 to 11. In order to reduce the overlapping of isolates, each of the 500 isolates was subjected to 12 different antibiotics. The strains were distinguishable on the basis of all the 12 different antibiotics. The response of the various isolates to antibiotics reveals that they differ in their tolerances abilities. Only one representative of each group was selected and 117 isolates were processed further. The results of distribution also help us to sort out and segregate the strains by comparing strains with each other.

The next aim was to test the hydrolyzing abilities of microbial isolates. Different isolates were patched individually onto selective media such as nutrient agar (as control), skim milk agar (1%), tributyrin agar (1%) and starch agar (0.2%), to identify their abilities to produce protease, lipase and amylase, respectively. Strains were sorted out on the basis of six different criteria and arranged in descending order based on high zones of hydrolysis and relative enzyme activities. Further these strains were recategorized into a group which had high values for atleast two enzyme activities. The net result of such an arrangement was fishing out of eight best strains.

Further polyhydroxyalkanoates producing abilities of microbial isolates were checked. More than hundred strains were screened for polyhydroxybutyrate production. Sixty were found to produce varying amounts of PHB. Thirteen strains could produce PHB in the range of 320 to 565 mg/L with the corresponding yields of
40 to 87%. Maximum number of isolates with a wide range of PHB producing abilities belonged to the genus *Bacillus*. Thirty-eight *Bacillus* strains were screened out of which 6 strains outperformed others by producing PHB in the range of 320 to 565 mg/L of PHB. Some of the selected strains were further biochemically characterized different properties like utilization of thirty three different carbohydrates as carbon source, five proteins as nitrogen source and seven enzyme activities.

Finally, metabolism of biowastes by microbial isolates into polyhydroxyalkanoates was done using pea shells as the feed stock. In the first phase of testing with synthetic media (GM2) of known composition was undertaken. As per the plan of work, the percentage of synthetic media was then replaced sequentially by acidogenic slurry of pea shells. Further work was carried after the selection of six *Bacillus* strains and these were tested for their efficiency to produce PHB from waste biomass. Since waste biomass is a mixture of complex carbohydrate sources, firstly, all the strains were tested for their ability to utilize different sugars for their growth and PHB production. This work was biphasic in the sense that firstly the organisms were grown on minimal media supplemented with individual sugars and growth curves were plotted taking O.D. at 600nm as the measure of growth. Following this, PHB production media (GM 2) with individual sugars was used to further test for PHB production of each strain. PHB producing abilities of different *Bacillus* strains varied from 190 mg/L to 485 mg/L on glucose supplemented GM2 medium. PHB constituted 31 to 62% of the total DCM.

In the next phase of testing, of the 6 chosen organisms, pea shell slurry in combination with GM2 media was used for production of PHB. Various parameters were changed (inoculum size, supplementation with external additional source of nitrogen etc) and their respective effects on PHB production were recorded. Since the overall objective is to maximize the contribution of biowaste in the feed, we checked the abilities of the six *Bacillus* strains on higher BW:M ratio of 1:1 and 7:3. Increasing the contribution of waste from 30% to 50% i.e. BW:M :: 1:1 resulted in 13 to 24% increase in PHB production with *B. cereus* EGU520, *B. cereus* EGU43 and *B. thuringiensis* EGU45. The effect of nitrogen supplementation on PHB production from biowaste was initially checked at the rate of 0.02% w/v of CEH. It was found to greatly enhance the PHB production and yields to various extents. At higher
contribution of biowaste in feed, supplementation with CEH proved effective up to BW:M :: 1:1 level. The trend of improvement in PHB production could not be sustained at BW:M :: 7:3 level in spite of CEH supplementation. The effect of concentration of CEH supplementation as nitrogen supplement seems to be very clear-cut on PHB production by *Bacillus* strains. Maximum enhancement in PHB production was observed with 0.4 g/L (0.04% w/v) supplementation, where the PHB production achieved was 1945 to 2075 mg/L and a yield of 71 to 72% w/w. An increase in inoculum size from 10 mg cell protein/L to 100 mg cell protein/L of feed has helped to improve the PHB production to 3010 to 3370 mg/L i.e. an improvement of 32 to 42%. The uniqueness of the strains tested in the last phase of experimentation lies in the fact that these strains have not been reported for PHB production so far. Production of PHB varied from strain to strain on glucose supplemented GM2 medium. The optimization of ratio of amount of BW:M combinations (1:1), addition of supplemental nitrogen source i.e. casein enzyme hydrolysate (at the rate of 0.4g/L) and inoculum size (10 mg cell protein/L) was done previously. All six strains were tested on these conditions and PHB production was recorded at 4 different time intervals. A common trend of steady improvement in PHB production was seen with increasing time with all the strains. Out of these 6 strains tested again on Biowaste for PHB production, the highest producers were *Alcanivorax* sp. EGU619 and *Myroides odoratus* EGU882 that could produce PHB to the level of 1015 and 1145 mg PHB/L respectively at 96h.