Summary and Future prospects
SUMMARY

- *Bacillus* spp. isolated from the marine and coastal environments were used for the study.

- These isolates exhibited temporal variation in their PHA accumulation pattern. Based on the PHA content accumulated within the cells, the isolates could be categorized into four groups.

- Isolates (BLQ-2/A7, COL1/A6, L2/A1 and L4/A4) belonging to one of these groups were capable of consistently maintaining the accumulated PHA without significant intracellular degradation during the entire experimentation time. This property is crucial in large scale PHA production as a variation in the time of harvest will not adversely affect the product yield.

- The *Bacillus* spp. used in this study were found capable of utilizing various carbon substrates for polymer production and exploitation of this ability resulted in the selection of some agro-industrial by-products (such as molasses, starch based residues, citrus pulp waste, coconut oil cake, bagasse and rice chaff) as potential carbon feedstock for PHA production.

- Majority of the isolates were capable of producing PHA using the agroindustrial substrates tested.

- Dilute acid hydrolysis of the agroindustrial by-products not only improved the ability of the isolates to assimilate the released fermentable sugars as PHA but also avoided the interference caused by insolubles present in the wastes during downstream processing when cultivated under submerged fermentation conditions.
• Isolates exhibiting maximum PHA fluorescence intensity on respective substrates when stained with Nile blue A were selected for further studies on PHA production under submerged cultivation conditions.

• Using molasses as the carbon source, all the isolates tested were able to accumulate PHA ranging between 51.23% and 68.56% DCW. Starch based residue was also efficiently utilized by the isolates as more than 55% DCW PHA was produced within the cells. Citrus pulp waste was able to support a PHA content ranging between 38.87% and 48.86% DCW in the Bacillus spp. tested. Coconut oil cake also served as a potential carbon source for PHA production with a highest PHA content of 42.4% DCW (COL1/A11).

• Isolates COL1/A6 and BLQ-2/A7 were selected for further based on their maximum and consistent production of PHA and ability to utilize diverse agro-industrial by-products.

• Optimization studies revealed that both the isolates were able to accumulate PHA between 20 to 40°C with the optimal temperature for growth and maximum PHA accumulation at 30°C.

• Strain COL1/A6 and BLQ-2/A7 exhibited pH optima of 7.0 for growth as well as PHA production. These isolates were able to grow and accumulate significant amounts of PHA in fermentation medium adjusted to pH values either one unit above or below their pH optima.

• Among the various ammonium salts tested, both the isolates produced highest PHA yields using ammonium chloride as a nitrogen source.
- Optimization studies carried out using different yeast extract concentrations indicated that a lower concentration range between 0.1 g L\(^{-1}\) to 0.5 g L\(^{-1}\) was ideal for PHA production. Further increase in the concentration to 5 g L\(^{-1}\) resulted in enhancement of growth only.

- Growth of the isolate BLQ-2/A7 under optimized conditions revealed that the isolate grows rapidly with maximum PHA of 59.42% DCW obtained at 48 h. However, nearly half of the sugar supplied was found unutilized in the medium at the end of the fermentation run.

- In the case of isolate COL1/A6, time course studies using batch cultivation showed that the isolate grows slowly but accumulates more amount of PHA (65.51% DCW) than isolate BLQ-2/A7 with only 25% of the supplied sugar left in the medium after the fermentation run.

- Studies on the improvement of the PHA yield were investigated with isolate BLQ-2/A7 since this isolate grows rapidly and is unable to efficiently utilize the sugar supplied.

- The model obtained employing response surface methodology indicated a positive interaction between glucose and ammonium chloride in case of biomass as well as PHA production. The studies also indicated that the concentration of glucose and ammonium chloride is critical for growth and PHA production.

- The conditions described for fed-batch I fermentation revealed that the addition of glucose and ammonia at 18 h of incubation created conditions conducive for biomass production rather than PHA formation.
• The fed-batch II strategy of fermentation (addition of ammonium at 12 h) resulted in an increase in the PHA content. Maximum content of 65.51% DCW within 24 h was achieved and the PHA content was found to be relatively stable for another additional 24 h.

• Fed-batch fermentation studies highlighted the importance of a simple nitrogen feeding strategy for improved substrate utilization and PHA production. The PHA yield using the isolate BLQ-2/A7 increased from an initial of 56.2% DCW at 48 h under non-optimized fermentation conditions to 65.5% DCW at 24 h by optimization of batch fermentation conditions coupled with a simple nitrogen feeding strategy.

• Screening of various non-solvent and solvent systems for the solubilization of cell material containing PHA revealed that treatment with sodium hypochlorite and sulphuric acid (4 h) resulted in the recovery of the polymer (purity 97.39% and 94.93% respectively). Among the solvents, chloroform proved to be a better solvent for solubilization of the polymer. Based on FT-IR profiles of the polymer obtained using various treatments, the best method was found to be treating the cells with sodium hypochlorite followed by acid treatment (4 h) and chloroform.

FUTURE PROSPECTS

The present study indicates the potential of *Bacillus* spp. as PHA producers. In this study, *Bacillus* spp. were able to unequivocally produce PHA ranging from 29.45% DCW (COL2/A2 on coconut oil cake hydrolysate) to 68.56% DCW (ICP-1/A3 on molasses) from low-cost agroindustrial residues. Further characterization of the produced polymer is necessary as this genus is known to produce PHAs with different
monomer composition from a wide variety of substrates (Valappil et al., 2007a; Tajima et al., 2003; Labuzek and Radecka, 2001). Based on these investigations further studies are envisaged for

- PHA production and high-cell density fermentation with intensive studies on fed-batch mode of operation involving pH, dissolved oxygen (DO) regulated system and glucose supplementation need to be investigated.

The high-cell density is vital for the economic viability of the production process (Madison and Huisman, 1999). The advantages of this cultivation system include higher product concentration, reduced investment in equipment, decreased production costs and increased volumetric productivity.

- Use of solid state fermentation (SSF) for production of PHA.

This is another attractive and emerging fermentation strategy that can be employed. SSF allows the use of inexpensive feedstock such as agro-industrial residues.

The advantage of employing such fermentation strategy provides solutions to the disposal of these residues with the simultaneous production of value-added products. The added advantage is that these residues can be directly incorporated in the fermentation media without any pretreatment necessary unlike submerged fermentation (SMF). The fermented solids containing PHA products can be used directly without downstream processing to prepare composite materials of increased biodegradability (Castilho et al., 2009).