Introduction

Plastics are utilized in almost every manufacturing industry ranging from automobiles to medicine. Most of the plastics and synthetic polymers are produced from petrochemicals, which are derived from fossil fuels. The focus has been diverted from synthetic polymers towards biopolymers, because of two major reasons. Firstly they cause environmental pollution and secondly they are derived from fossil fuels. The reservoir of fossil fuels is now reaching its bottom line, which will lead to an increase in the production cost of synthetic polymers.

It is a well-acknowledged fact that commercial use of biodegradable polymers will greatly reduce the increasing dependence on synthetic polymers and its environmental impacts. Polyhydroxyalkanoate (PHA) is an intracellular microbial thermoplastic that is widely produced by bacteria present in soil (Anderson and Dawes, 1990; Page, 1995). PHAs can be produced in bacteria, recombinant bacteria and genetically engineered plants. The preferences of using a microbial source for large-scale production of biodegradable polymers can be advocated because of the advantages of rapid mass production and malleability of bacterial genetic makeup. Also, bacteria have been found to integrate novel monomers in the polymer, which increases the diversity of PHAs produced. There is still ongoing interest to find novel PHAs with novel constituents or a new combination of already known constituents.

Owing to their biocompatibility and biodegradability, they are receiving an increasing attention for their commercial application as substitutes for synthetic plastics. The applications of PHAs are not limited to packaging consumables but extend to quality applications in medicine and pharmacy and nanocomposites in biomedical devices (Zinn et al., 2001, Chen and Wu, 2005; Pandey et al., 2005).

The presence of stress in form of nutrient limitation or presence of xenobiotics leads to physiological response in the form of unbalanced growth leading to an increase in the PHA content. It is thought of as a strategy to increase survival and stress tolerance in changing environments (Ayub et al., 2004; Kadouri et al., 2005). PHA producing bacteria have been repeatedly isolated from oil-contaminated soils (He et al, 1998; Tian et al., 2000; Hang et al., 2002; Haba et al., 2007). Oil-contaminated soils contain about 84% carbon, 14% hydrogen, 1-3% sulfur and less than 1% of nitrogen and other compounds (Atlas, 1995). Excess carbon with less than 1% nitrogen make these sites a potential source for isolating PHA producers since the synthesis of PHA is favoured by environmental stresses (Anderson and Dawes, 1990;
Several recent studies have reported the isolation of PHA producing bacteria from oil contaminated sites (He et al., 1998; Tian et al., 2000; Hang et al., 2002; Haba et al., 2007). However, our understanding of diversity of PHA producers at oil contaminated sites remains limited. Further, present literature on PHA producing bacterial strains is limited to a relatively small number of well characterized bacterial genera. Due to this, from oil contaminated sites, producers belonging to the Pseudomonas genera have been rediscovered in different studies. Hydrocarbon-degrading bacteria that can produce PHA have an extended advantage of degrading recalcitrant pollutants and production of a commercially important polymer from them.

The first objective of this research was therefore to investigate the presence of PHA producing bacteria in the oil-contaminated soils in India. A polyphasic approach comprising of phenotypic and genotypic based screening was done for identifying the bacterial strains that could accumulate PHA.

In spite of the advantages of PHAs compared with petroleum-derived plastics, their use is currently limited due to their high production costs (Kellerhals et al., 2000). A significant portion of the cost associated with PHA production is the cost of the substrate used for growth and polymer accumulation. One approach to reduce the cost of PHA is to use inexpensive carbon source. Alkanoates such as octanoate are the best carbon sources for all MCL-PHA synthesizing Pseudomonas. However, using pure alkanoates for MCL-PHA synthesis increases the production costs of these polymers. Considering that pure fatty acids are expensive, vegetable oils and their fatty acids have greater suitability for production of MCL-PHA economically (Lee et al., 2000).

The next objective was therefore to find substrates that would reduce the production cost of MCL-PHAs by the selected bacterial strains.

Investigations have also focused on reducing the total cost of PHA by optimizing fermentation process using statistical methods. Medium optimization for overproduction of any metabolite is an important step for its commercial use and involves a number of physicochemical parameters. This is an effective and powerful approach for screening key factors rapidly from a multivariable system to optimize fermentation conditions and have been extensively used recently. Several studies have been carried out with various micro-organisms and fermentation processes for production of PHA (Fukoi and Doi, 1998, Fernandez et al., 2005). But a statistical design of experiments was not applied for optimization of the media components. Therefore, scattered and different concentrations of media components have been reported in the literature.

The other objective was to apply statistical design of experiments for designing and optimizing fermentation medium for MCL-PHA production by the selected bacterial
strain. For this purpose, significant medium components were first screened and selected on the basis of the 2-level Factorial design. Subsequently, the selected variables were optimized by RSM using Central Composite Design (CCD).

The aim of this work was to investigate the presence of PHA producing bacterial strains at different oil contaminated soils in India and to enhance the synthesis of PHA production in the bacteria strain isolated from oil contaminated soil.

- Screening bacterial strains isolated from oil-contaminated sites for PHA production using a phenotypic and a genotypic method.
- Phylogenetic analysis of the *phaC* gene in selected strains.
- Screening of economically viable substrates for production of PHA by selected bacterial strains.
- Optimization of physiological and nutritional parameters using one at a time method and response surface methodology
- Upscale of PHA production by selected bacterial strain under optimized conditions.
- Downstream processing from the fermented broth.

This thesis is divided into the following nine chapters:

**Chapter 1: Introduction**

The chapter includes the information, which is useful to decide the main objective of the work. This chapter will give broad overview of microbial diversity of PHA producers, applications and economic importance of PHA production.

On the basis of the background, the objectives of the research work are also listed in this chapter.

**Chapter 2: Review of Literature**

This chapter will present an overview of the previous work and knowledge about the PHA producing bacteria, their diversity, inexpensive substrates and statistical tools to study optimization of fermentation conditions. The chapter will be divided in following sections to cover all the aspects.

- Historic Preview
- Structure, Properties and types of PHA
- Applications
- Environment, Phylogeny and Diversity of microorganisms producing PHA
Introduction

- Methods for detection and visualization of PHA
- Advances in MCL-PHA production from inexpensive substrates by Pseudomonads
- Effect of Physiological and nutritional parameters on PHA production
- Statistical design experiments

Chapter 3: Materials & methods

This section will provide details of all the experiments done to achieve the above mentioned objectives. The details of material/chemicals used and protocols followed for each experiment will be clearly explained. Hence this chapter will explain all the techniques used for screening and selection of bacteria for PHA production, study of effect of ecological factors and fermentation optimization.

Chapter 4: Results

This chapter will be presented in the following sections. The summary of the results obtained is mentioned below.

Screening and selection of potential PHA producing micro-organisms

This section describes the methods used for screening bacterial strains for PHA production capability. The selection of most efficient bacterial strains is also explained here.

Phylogenetic analysis of the phaC gene fragment in the selected bacterial strains

This section describes the determination of the phaC gene involved in PHA production using a set of degenerate primers. This section also describes the gene sequence and its phylogenetic position.

Optimization of nutritional parameters for PHA production from strain Brochothrix thermosphacta TERI 5001

This section describes the use of “one-at-a-time” method and for PHA optimization by the selected bacterial strain.

Optimization of nutritional parameters of strain Pseudomonas aeruginosa TERI 13012

This section describes the use of “one-at-a-time” method and response surface methodology (RSM) for PHA optimization by the selected bacterial strain.
Up scaling of the fermentation process

This section describes the scaling up of the fermentation process in 100 litre working volume.

Analysis of PHA

This section describes the GC-MS and NMR analysis of PHA extracted from the selected bacterial strain.

Chapter 5: Discussion

The results obtained in this study will be discussed in detail using the available literature.

Chapter 6: Summary

This chapter will summarize the research and its implications.

Chapter 7: References

Chapter 8: Annexure

Chapter 9: Publications